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PROTOGASTER, REPRESENTING A NEW ORDER OF THE GASTEROMYCETES[1]

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A most interesting Gasteromycete was discovered by the late Dr. Roland Thaxter at Kittery Point, Maine, August, 1895. Dr. Thaxter applied to this fungus the generic name, *Protogaster.* Its tiny subspherical fructifications were found from one to three inches below the surface of the ground, attached to or "running from the roots of the cultivated pansy." In a letter to the writer under date of March 15, 1932, Dr. Thaxter said "The fungus seems a very curious and interesting one. It appeared to be definitely a parasite on the roots of Viola." It is, indeed, an interesting Basidiomycete!

In one collection (*5661*) Dr. Thaxter had preserved intact a clod of soil glued to the bottom of a small pill box. Nestled in some of the crevices and hollows of the clod are groups of the basidiocarps of this fungus (pl. 7). Under the low-power binocular microscope these fructifications have the appearance of tiny cocoons of some insect or the egg-sacs of tiny spiders. They adhere to the soil or to roots by minute cobwebby hyphae which radiate in every direction from the sporophore. These

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are so numerous from the surface of very young fructifications that the surface of the latter is nearly obscured. In the more mature stages this indefiniteness becomes a more definite meshy surface. In pl. 7 such a group of sporophores in several stages of maturity is shown in close proximity to a small rootlet.

The three collections taken by Dr. Thaxter formed the basis of the studies here reported. The collections together total hundreds of the fructifications dried with some of the soil in which they were found. These gasteromycetous fruiting bodies are from 0.1 to 0.5 millimeter in diameter. The youngest ones are white, while the more mature individuals are pale brownish-drab, the surface being of a soft dry byssoid to innately fibrillose character. The loose hyphae of the surface of two or more fruiting bodies often intertwine, causing them to adhere to each other. When several are involved a bead-like series may result. This adherence, however, in all the observations made by the writer involves only the superficial hyphae of the peridium, and the internal structure remains entirely separate. Each of the bodies in these cases remains a morphologically distinct entity.

In order to study the morphology in detail several fructifications were prepared and microtome sections were made. They were put through the formal-acetic-alcohol fixative and stained with safranin-gentian violet. When saturated by a clear solution, such as formal-acetic-alcohol, the sporophores with immature spores changed merely to a creamy white, while the more mature ones having the glebal locule stuffed with spores changed immediately to a color very close to vinaceous-fawn.

The prepared slides and crushed mounts show the peridium to be a simple layer of meshy or loosely interwoven hyaline hyphae (prosenchyma) which are white to cream-colored (*sub lente*) and usually are considerably branched. The layer is 17–46 μ thick. This comprises the whole of the sterile tissue of the fruiting body except the subhymenial layer which is composed of compacted hyphae taking a deeper stain than the peridium or hymenium. This subhymenial layer is very thin, usually the thickness of two or three hyphae (2–4 μ thick).

The gleba is extremely simple, consisting of one spherical locule, the wall of which is a definite smooth hymenium composed of clavate to fusiform basidia interspersed among short-clavate paraphyses. The basidia are mostly 1–4-spored, 2-spored basidia predominating in young fruiting bodies. The basidia are 8.5–11 x 3–7 μ. In cases of large accumulations of spores at maturity the gleba in general appearance assumes a clay color or Mikado brown to snuff-brown. Where compact accumulations of spores are found the spore mass assumes a color darker than indicated above.

When considered in terms of the capacity of the locule, spores are produced in large quantities. At maturity the cavity is completely filled, and there is some indication that the pressure of the spores may burst the peridium, liberating the former. As a rule the peridium doubtless disintegrates in the soil, with spore dispersal by water and possibly by insects.

The spores are most commonly pip-shaped to obovoid, but are sometimes ellipsoid with broadly rounded ends. Under the microscope they are hyaline to dilute citrinous but in mass they are from clay color to Mikado brown or snuff-brown. The exospore is rather thick and distinct. The spores measure 8–12.5 x 3.5–6 μ.

This is doubtless the most simple gasteromycetous form yet discovered. The tiny subspherical sporocarps with subterranean origin and life cycle are very similar in fundamental tissues and ecological conditions (hypogeous) of development to those of the button stages (primordia) of the sporophores of more highly differentiated Gasteromycetes and angiocarpous Agaricales. The primordia of the sporocarps of the plurilocular Gasteromycetes and of the Agaricales for the most part have a hypogeous origin but further developmental stages, except for most of the Hymenogastrales, emerge to an epigeous stage at maturity. With such a recapitulation of this button-like hypogeous form in the primordial sporocarps of most of these higher fleshy fungi it would not be difficult to postulate the emergence of the epigeous forms among them from such simpler hypogeous forms as *Protogaster*.

The peridium of *Protogaster* represents all of the sterile tissue of the normal sporophore. It is a simple layer of undifferentiated prosenchyma of a rather loose mesh, the remains undoubtedly of the fundament of the developing fructification. This type of peridial structure perhaps should be interpreted as the most primitive "universal veil" or volvate tissue of the Gasteromycetes, and it can perhaps with equal truth be said to be the forerunner of the volva (blematogen) in the primordium of the angiocarpous development of certain of the Agaricaceae.

The mycelioid hyphae which radiate into the soil from the sporophore usually are not organized to any degree into rhizomorphic cords. Two, three, or four hyphae may twine loosely together to form a meshy strand, but they seem never to assume such a definite relation and close contact with each other as are maintained in an organized rhizomorph. Most, if not all, of the previously known Gasteromycetes have well-developed fibrils or rhizomorphs at the termination of certain of which the fructifications are borne.

A study of the dry herbarium material of the fungus under consideration gives little clue as to its orientation *in situ*. For the most part the fructifications are spherical or oblong, and there is *usually* no definite place of attachment discernible. In a very small percentage of basidiocarps studied there is a condition, however, which must not be overlooked in this connection. Out of twenty-seven fructifications which were studied critically, three showed the formation of a peg-like growth from the peridium into the locule. In each of these cases there was found a very inconspicuous place of attachment just below this exceptional peg-like growth. Sixty-eight additional specimens were examined *in toto* by transmitted light, by which two more were found with the growth. The peg-like growth is of the same loose structure as that of the peridium. It is not an invagination of the peridium but should doubtless be more properly interpreted as a mere tendency toward further sterile tissue. It is perhaps in fact a tendency toward a very rudimentary sterile base or even the rudiment of a columella. The one of these of which the writer has pre-

pared microtome sections extends into the locule 67 μ and is 42 μ in diameter at the base. This particular fructification is 374 μ in diameter and the locule measures 292 μ in diameter. This rudimentary columella (?) or sterile base (?) is not a constant morphological character of *Protogaster* and thus it has not been incorporated in the formal description below.

All previously described Gasteromycetes are plurilocular and therefore involve many more sterile elements, such as plates and septa in the gleba, than found in *Protogaster*. Since *Protogaster* is so unique in its morphological simplicity and unilocular character of the gleba, a new order and family are proposed to include this genus. The new order is proposed to comprise all unilocular Gasteromycetes having no specialized sterile tissues, and the family to include all of those of the new order which show no invagination, have a definite even hymenium.

Dr. Thaxter gave the genus name, *Protogaster* (*in Herb.*), and in his search for a suitable specific name he had used three, to-wit, *P. rhizophilus, P. radicicolus,* and *P. Violae.* The writer has adopted the specific name which appears (*in Herb.*) on the collection having the lowest accession number. The diagnostic description follows:

PROTOGASTRALES Zeller, ord. nov.

Fructificationes subsphaericae, sine speciale sterile textu; peridium indehiscens; gleba uniloculata.

Fructifications spherical to subspherical, with no specialized sterile tissues present; *peridium* simple, closed; *gleba* unilocular.

PROTOGASTRACEAE Zeller, fam. nov.

Fructificationes subsphaericae, minutae; *peridium* simplex contextum, indehiscens; *gleba* uniloculata, sine invaginatione; hymenium laeve.

Fructifications small, subspherical; *peridium* of simple fundamental tissue, indehiscent; *gleba* unilocular, showing no invagination; *hymenium* smooth.

PROTOGASTER Thaxter, gen. nov.[1]

Fructificationes minutae, sphaericae vel subsphaericae, sine speciale sterile textu, hypogeae; peridium simplex primordiale contextum, indehiscens; gleba uniloculata, sine invaginatione; hymenium laeve; sporae ellipsoideae vel subellipsoideae, laeves, hyalinae vel subcoloratae.

Fructifications small, spherical to subspherical, with no specialized sterile tissues, hypogeous; *peridium* of simple fundamental tissue, indehiscent; *gleba* unilocular, showing no invagination; *hymenium* smooth; *spores* ellipsoid to subellipsoid, smooth, hyaline to slightly colored.

Protogaster rhizophilus Thaxter, sp. nov.

Fructificationes sphaericae vel ellipsoideae, 100–500 μ diametro, hypogeae, superficie arida, alba vel pallide brunneo-grisea, byssoidea vel innato-fibrillosa (sericea), unde hypoidei funiculi in soli evanescunt; peridium 17–46 μ crassitudine, simplex, byssoideum, ex hyphis tenuibus laxis undulatis; gleba uniloculata, "clay color," "Mikado brown," vel "snuff-brown";[2] locellus subglobosus, primo vacuus, maturitate sporis repletus; hymenium laeve; basidia inconspicua, hyalina, clavata vel fusoidea, mono- vel tetraspora, vulgo dispora, 8.5–11 x 3–7 μ, sterigmatibus brevibus; sporae subellipsoideae vel obovoideae, laeves, hyalinae vel dilute citrinae *sub lente, in massa* "Mikado brown" vel "snuff-brown," exosporo subconspicuo, 8–12.5 x 3.5–6 μ.

In terra inter radices vivas Violae, Kittery Point, Maine, Amer. bor. (*Roland Thaxter*). Aestate.

Type: Kittery Point, Maine, August, 1895, *Roland Thaxter* (in Thaxter Bequest (No. 5660) to Farlow Herb., Harvard University).

Fructifications spherical to ellipsoid, 100–500 μ in diameter, hypogeous, held in soil cavities by cobwebby hyphae radiating in all directions from the peridium; *surface* white to pale brownish drab, dry, byssoid to innately-fibrillose (silky); *peridium* simple, of loosely interwoven hyphae mostly parallel with the surface, hyaline, 17–46 μ thick; *gleba* unilocular, from clay color to Mikado brown or snuff-brown due to accumulation of spores; *cavity* subglobose, empty at first but stuffed with spores at maturity; *hymenium* smooth; *basidia* inconspicuous,

[1] The writer assumes the whole responsibility for the description of the genus and species. Dr. Thaxter left no manuscript with the herbarium material and had published nothing pertaining to *Protogaster*.

[2] Ridgway's 'Color Standard and Nomenclature' was used throughout this paper.

hyaline, granular, clavate to fusiform, 1–4-spored (2-spored predominate in very young fruiting bodies), 8.5–11 x 3–7 μ; *spores* pip-shaped, obovoid, or subellipsoid with broadly rounded ends, smooth, hyaline to dilute citrine (*sub lente*), Mikado brown to snuff-brown *en mass,* exospore rather prominent, 8–12.5 x 3.5–6 μ.

One to three inches below the surface of the soil surrounding living roots of the cultivated pansy (*Viola*), Kittery Point, Maine (*Roland Thaxter*). August.

Specimens examined:

MAINE: York County, Kittery Point, August, 1895, *Roland Thaxter, 3282, 5660* (type), *5661* (in Thaxter Bequest to Farlow Herb., Harvard University).

Since the class, Gasteromycetes, is generally recognized as a grouping of several apparent series or lines of parallel or perhaps divergent tendencies in development, it cannot be considered as a natural single division of the fungi. For this reason it cannot be said that *Protogaster* is more primitive because of its greater simplicity than all forms exhibiting the several series of developmental tendencies in the previously described Gasteromycetes. Rather, it seems to take its place in one of the lines, namely, that with smooth ellipsoidal spores and with also a definite smooth hymenium. It seems to the writer that the most natural connection is from *Protogaster* through species of *Rhizopogon,* with their hypogeous nature, and finally through the Lycoperdales.

Connecting links may yet be discovered between *Protogaster* and *Rhizopogon.* And, too, other new forms may in time more definitely indicate the possible point or points of divergence of more primitive lines of development branching off from this main (?) trunk below *Protogaster.*

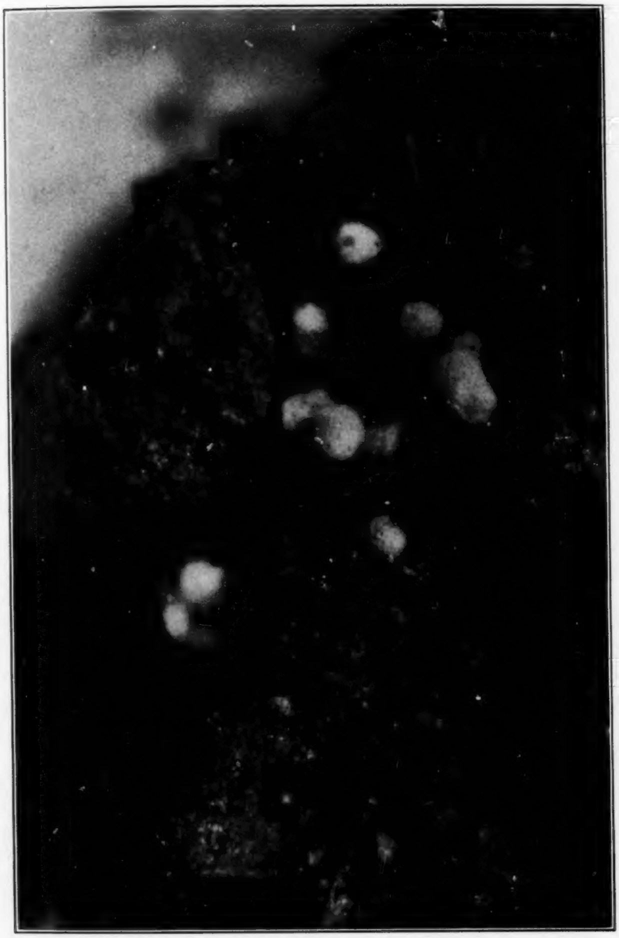
The writer is gratefully indebted to the late Dr. Roland Thaxter for the privilege of studying this most interesting fungus and to Professors J. H. Faull, W. H. Weston, Jr., and Oakes Ames, committee administering the Farlow Herbarium, for their cooperation in putting the herbarium material at his disposal.

Explanation of Plate

PLATE 7

***Protogaster rhizophilus* Thaxter**

Sporophores in a crevice and hollow on the surface of a clod of soil taken 2 to 3 inches below ground, Kittery Point, Maine, August, 1895. (*Roland Thaxter*). About × 32. Photograph by Dr. Frank P. McWhorter.



ZELLER—PROTOGASTER

Explanation of Plate

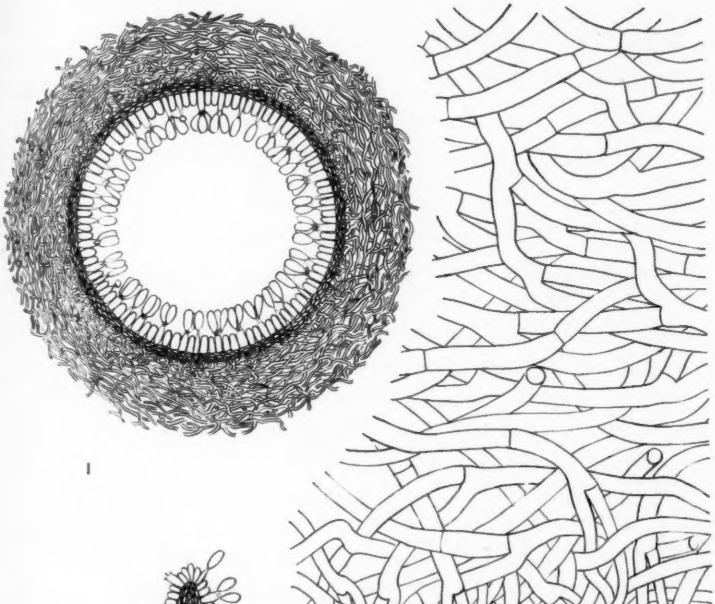
PLATE 8

Protogaster rhizophilus **Thaxter**

Fig. 1. Diagrammatic median section of a basidiocarp, illustrating the relation of morphological parts. × about 300.

Fig. 2. Diagrammatic sketch of the peg-like growth of fundamental tissue rarely found to extend into the locule. × about 300.

Fig. 3. Drawing to scale of a section through the peridium and subhymenial layer, showing the relation to basidia and paraphyses, highly magnified. × about 3000.



ZELLER—PROTOGASTER

CERTAIN PHYSICAL AND STRUCTURAL PROPERTIES OF THREE SPECIES OF SOUTHERN YELLOW PINE CORRELATED WITH THE COMPRESSION STRENGTH OF THEIR WOOD[1]

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There is a growing demand among lumber consumers for maximum strength values per unit volume of material and more reliable working stresses for structural timbers. This situation, allied with the rapid depletion of virgin growth timber, has made it difficult for the lumberman to meet the requirements of commerce without a more perfect knowledge of the factors influencing the strength and usability of wood than he has had in the past. Numerous researches, beginning with that of Parent about the year 1707, have been made on the mechanical properties of wood. Fernow ('92) summarized the earlier works, and the more recent investigations have been covered by The National Committee on Wood Utilization ('29) and by Withey and Aston ('30).

Defects in the form of knots and shakes have a definite effect upon the strength of beams, depending upon the size, condition, and position of the imperfections (Newlin and Johnson, '24). Spiral- and cross-grain have a decided effect upon strength and elastic properties, depending upon the angle at which the fibers lie in relation to the axis of the test stick (Wilson, '21).

The strength properties of clear wood have a certain relation to the specific gravity (density) regardless of species (Newlin and Wilson, '19, and Markwardt, '27), but it is readily observed

[1] An investigation carried out jointly in the Graduate Laboratories of the Henry Shaw School of Botany and the Department of Civil Engineering of Washington University and submitted as a thesis in partial fulfillment of the requirements for the degree of doctor of philosophy in the Henry Shaw School of Botany of Washington University.

[2] A fellowship established by the American Creosoting Co.

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that this relationship has definite limitations. For wood in general there may be a variation in compression strength of from 2000 to 3000 pounds per square inch for a given density. Wood taken from trees of the same species, and even from the same tree, has a variation of from 40 to 70 per cent (based upon the minimum strength) for a given specific gravity.

The purpose of this investigation on the wood of southern yellow pine was to determine the causes of these variations and if possible to describe them so that their presence might be recognized without first testing the material. It was found that the variations may be attributed to two sets of variables which, though interlocking, may be roughly outlined, as (1) the physical condition and chemical constituents of the cell wall, and (2) the anatomical structure of the wood. The physical conditions include the fiber-saturation point, the moisture content, the absolute density of the wood substance, and the percentage of resin affecting the specific gravity. Anatomical differences in the southern pines were the relative amounts of thick- and thin-walled fibers in a given area as determined by rate and type of growth, the size and distribution of resin ducts and wood rays, the length and diameter of the tracheids, and the structure of the cell walls. In evaluating the strength on the basis of structure, all specimens containing visible defects in the form of knots, checks, and shakes, and cross-grain of more than 1 in 25 were not used. A knowledge of the fiber-saturation point was necessary in order to eliminate moisture effects on strength. In addition, the specific gravity was corrected for benzol-soluble compounds in order to get the true relationship between the density and strength, since this was the most accurate measure of the relative mass of the different specimens. No attempt was made to study the chemistry of the cell wall.

MATERIALS

The seven trees used in this study were selected by representatives of the American Creosoting Co., and the writer is indebted to these men for the data to be found in table I. Three

species of southern yellow pine were included: *Pinus Taeda* L. (loblolly pine, trees 1, 2, 5, and 7), *Pinus echinata* Miller (shortleaf pine, tree 3), and *Pinus palustris* Miller (longleaf pine, trees 4 and 6).

One 32-ft. log was taken from the butt portions of trees 1, 2, 3, and 4, whereas three 8-ft. logs were taken in trees 5, 6, and 7, one at the butt, one from the top of the merchantable timber, and the third midway between the other two. In the latter group (trees 5, 6, and 7), note was made of the distance of each 8-ft. log from the stump. The seven logs collectively covered a range of 68 feet from the stump, so that the material was sufficient to make a comparative study of the distribution of strength throughout the merchantable timber.

Each of the logs was marked as to compass direction and tree number when cut, and samples of leaves and cones were collected from each tree for botanical identification. The ends were painted to prevent drying out, and care was taken to preserve the bark intact. They were shipped in the log to Philip Gruner Bros. Lumber Co., St. Louis, where they were sawed into test sticks for the laboratory. For purposes of identification, each log was considered as divided into 4-ft. lengths called bolts which were consecutively lettered *a, b, c,* etc., beginning at the butt. Bolt *a* in each tree represented the first 4 feet from the stump, *b* the second 4 feet (the segment between 4 and 8 feet), *c* the third segment between 8 and 12 feet, and bolt *q* represented the segment betwen 64 and 68 feet (fig 1a).

Each bolt was outlined on the larger end (fig. 1b) from north to south through the pith and from east to west, in order that 4 series of specimens across the entire stem in the two directions could be identified. The lines were drawn at intervals of two inches, which allowed room for sawing and dressing the sticks. The individual specimens were then marked by tree number, bolt letter, compass direction, and distance from the pith. From the pith to the bark on the north and south sides there were two series of specimens numbered 1, 3, 5, 7, etc., and 2, 4, 6, 8, etc., respectively (fig. 1b). The same was true for the east and west sides beginning with the second specimen from

the pith. Adjacent to each of these was a second series bearing sub-numbers. The latter series was available only in the larger trees. For example specimen 3eE3 from tree 3, bolt *e*,

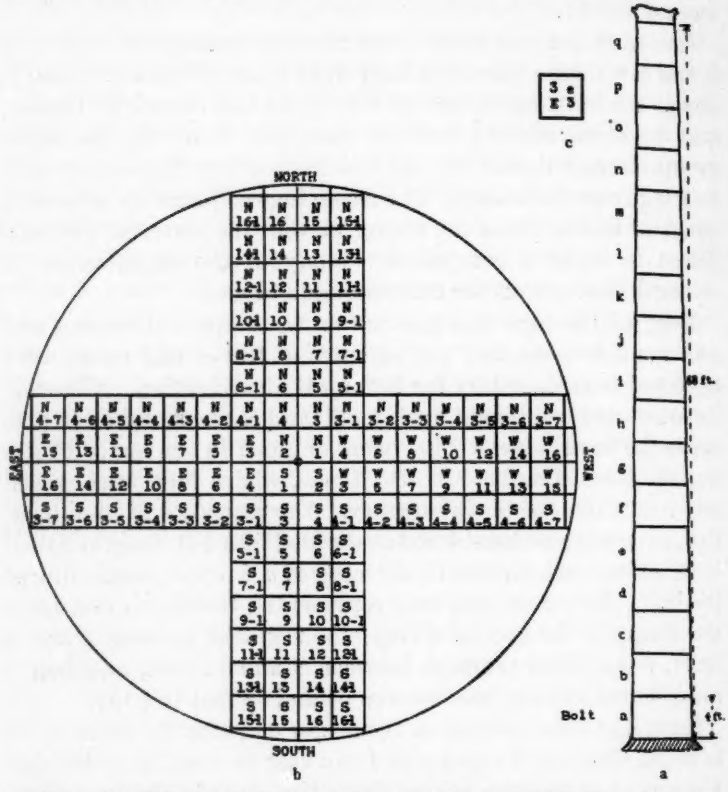

Fig. 1. A diagrammatic sketch showing the method of selecting and marking test specimens. *a*, the trunk of the tree divided into bolts; *b*, the cross-section of a bolt, showing the method of labeling; *c*, a typical specimen with complete markings. The specimen indicated was from tree 3, bolt e, on the east side, and was the second specimen from the pith.

on the east side of the tree, with its center 3 inches from the pith, would be marked as shown in fig. 1c. The first figure in any specimen number or identification mark refers to the tree

number, the first letter (which is always a small letter), to the bolt, the second letter (always a capital), to the compass direction, and the last figure or series of figures (as in 5bN3-3) gives the position along the radius or distance from the pith.

The method of marking specimens is in accord with the recommendations of the American Society for Testing Materials, except that the marking was done on the bottom or larger end of the bolt in order to save all specimens six inches or more in length in the outer sapwood.

The material tested green was surfaced at once and stored in wet sawdust in the laboratory during the period of testing. Moisture samples were taken from each test stick immediately on receipt at the laboratory in order to get a measure of the moisture distribution in the green tree. The material tested dry was allowed to season about three months in the rough, then surfaced and stored in the laboratory until tested. The material from trees 1 and 2 was kept in the laboratory for about a year before the tests were made.

Physical Properties

THE FIBER-SATURATION POINT

The fiber-saturation point of wood may be defined as the state in which the wood substance is saturated throughout without moisture existing as free water in the lumina of the cells or intercellular spaces. Theoretically, the physical properties of the wood fiber should not change with further additions of water since it would only displace the gases in the free spaces. Bearing in mind the fact that resins and oils present in the pine wood are not soluble in water, it can readily be seen that a perfect gradient of moisture, which is necessary in order to locate the exact point of saturation, is very difficult to obtain. The more common methods of measuring the fiber-saturation point are based upon either the change in volume of the specimen, the change in strength properties, or upon the change in specific conductance, all of which agree in general but vary over a wide range for any given specimen due to moisture gradients in the sample. The relationships of moisture-volume, moisture-

strength, and moisture-conductance all show a sharp change in rate at the fiber-saturation point. The advantage of the electrical measurements is that very small specimens can be used, and thus a more perfect distribution of moisture can be obtained.

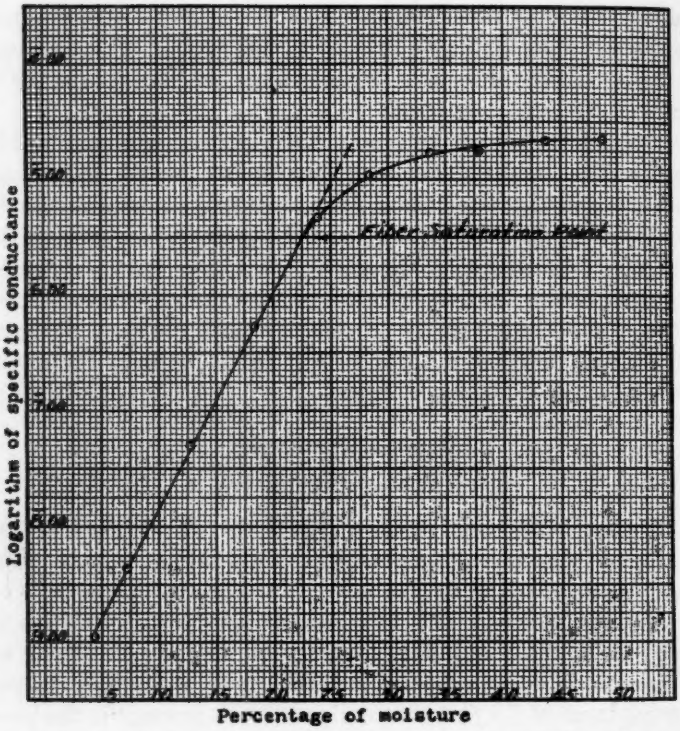

Fig. 2. The logarithm of the specific conductance plotted against the percentage of moisture. The sudden break in the curve indicates the fiber-saturation point, which was at 22.5 per cent moisture.

The fiber-saturation point reported here was obtained from electrical conductivity measurements made by the method introduced by Myer and Rees[3] and modified by Stamm ('29a). This method is known as the electro-conductivity

[3] Myer and Rees. 1926. Cited by Stamm ('29a).

method. The results obtained by it are based upon the assumption that the specific conductance of the wood varies directly in proportion to the increase in moisture from the oven-dry condition to the fiber-saturation point, where it ceases to increase in proportion to additional moisture. Therefore, when the logarithm of the specific conductance was plotted against the percentage of moisture, a straight line was obtained up to the fiber-saturation point, where it broke sharply to a curve (fig. 2).

Measurements made by this method are subject to considerable error, and accordingly only averages of a great number of individuals are of any value. The most outstanding variables were polarization and moisture gradients caused by the presence of resins. Stamm ('29a) stated that "the effects of possible polarization were found to be negligible because of the extremely low conductance measured." In these experiments this was not found to be true; polarization was strong in all cases, even with the lowest possible currents. Differences in the lengths of the specimens used also caused considerable variation in the conductance, even though they were kept in sealed bottles for twelve hours or more. Accordingly, the specimens used were kept as close to 1 mm. in length as possible.

The fiber-saturation point of the three species of southern yellow pine studied in these experiments was found to lie in the neighborhood of 22.5 per cent moisture. When the averages of smaller groups of data were plotted independently the fiber-saturation point varied somewhat, and the best interpretation that could be given to these results was 22.5 $\pm$ 1 per cent moisture. This is within the range reported by Tiemann ('06, '07) and Wilson, Carlson and Luxford ('30), based upon bending and compression strengths.

The difference between species was well within the range of the individual variations of any given species, but a sufficiently large number of individual measurements on the separate species might show a consistent difference. This number would necessarily be very great and on resin-free wood.

MOISTURE CONTENT

The moisture content of the test specimens was determined from 1-in. sections cut between the bending and compression specimens, and from 2-in. samples cut from the compression specimens. These sections were weighed immediately after the test specimens were cut, dried to constant weight at 95–100° C., and reweighed. In very resinous specimens these determinations were subject to some error. Resinous materials tended to evaporate at temperatures around 100° C. and occasionally the liquid resin dripped from the specimens while in the oven.

Tests were made on material from four different conditions of moisture: material just as it came from the green log, material seasoned in an open-air shed, material cured in the relatively warm laboratory, and oven-dried material. Only the tests made on green material and that seasoned in the laboratory are reported here.

In trees 1 and 2 *(Pinus Taeda)*, tested in the lab.-dry condition, the material had been stored in the laboratory for about a year, so it was very dry and for the most part of a uniform moisture content. Tree 1 averaged 7.1 per cent moisture with a range from 6.0 to 8.8 per cent. Tree 2 averaged 7.3 per cent with a range from 5.5 to 11.1 per cent (table VI).

In the green material little variation was found in the moisture content of the heartwood in any one tree, regardless of position. There were only a few specimens in the heartwood of tree 4 that were in the range of the fiber-saturation point. (See strength tables VII and VIII for the moisture data.) The *average* moisture content of the entire section has no particular significance due to varying amounts of sapwood and the sharp differentiation of moisture in heart- and sapwood.

ABSOLUTE DENSITY OF THE WOOD SUBSTANCE

The absolute density of pine wood fiber as reported here is the weight of a unit volume of the resin-free wood substance in relation to the same volume of water at 4° C. Measurements were made on sawdust by the pycnometer method at a constant

temperature of 30° C. The sawdust was taken from various positions in the tree and from each of the different species. It was first dried and treated for some time in benzol, which was kept warm in an oven, to extract the resin and other soluble compounds. The benzol was then removed with alcohol and the sawdust dried to constant weight at about 80° C. Next, it was boiled for some time in water, and a vacuum of about 25 cm. was applied at intervals to remove the air. This was continued until the air was replaced by water and the bottle reached a constant weight at 30° C. A range of values from 1.5156 to 1.5273 was found, with an average of 1.52 obtained by dropping the last two decimal places. The absolute density of southern yellow pine wood substance is thus given as 1.52.

Stamm ('29b) reported the density of *Pinus Taeda* as 1.531 (pycnometer method) when he used water, and 1.466 with benzol. Dunlop ('14) reported a density of 1.6197 for *Pinus palustris* by using acid and of 1.5060 with water, but since all of his results varied considerably he suggested 1.54 as a mean to represent in a rough way wood substance from all species.

PERCENTAGE OF RESIN

The percentage of benzol-soluble compounds designated as resins was determined by the loss of weight of oven-dried sawdust after soaking it for some days in benzol. The sawdust was taken from the compression and specific gravity specimens.

There was a certain relationship between the number of resin ducts and the percentage of resin, but no consistent correlation. The percentage of resin was greatest in the first 2 to 4 inches from the pith, often decreasing very sharply and then increasing again in the outer sapwood (tables VII and VIII). Resin was also abundant in and around injuries and knots, but it was often in localized areas where there was no sign of injury or knots and no increase in number or size of resin ducts. The percentages of resin reported here are in close agreement with the results given by Gomberg ('93), who made a more or less thorough study of the resin of *Pinus palustris* from Alabama.

SPECIFIC GRAVITY

The specific gravity was determined from 6-in. samples cut from between the bending and compression specimens in trees 1–5 and from 2-in. samples cut from the compression specimens in trees 6 and 7. There was less variation in the density-strength relations in the latter two trees. In the green material the samples were weighed in air and in water, dried to constant weight and reweighed. The specific gravity was calculated from the green volume and the oven-dry weight. The seasoned specimens were weighed in air and in water as above, but the sap pieces were reweighed in air, after being wiped off with a cloth, in order to determine the change in weight due to absorption of water while submerged.

It was found that sapwood specimens absorbed from 3 to 10 per cent of water during the process of weighing. It can readily be seen that the specific gravity thus obtained was subject to a certain amount of error. This error was diminished by reweighing but it may still be sufficient to affect the density-strength relations.

The presence of resin increased the specific gravity considerably, and where the resin was in localized areas or pockets the change in density was very sudden with no corresponding change in strength. In the case of very resinous material, the specific gravity was more than 0.1 too high, and in extreme cases more than 0.2. Only the corrected values were used in the interpretations of strength and structure in this paper.

Since the specific gravity of a particular specimen increased with a decrease in moisture, due to shrinkage below the fiber-saturation point, only specimens of about the same moisture content should be compared. Since the fiber ceased to expand with additional moisture above the fiber-saturation point, specific gravity values for green wood were especially comparable where they were corrected for resin.

The specific gravity in trees 1, 2, 3, 5, 6, and 7 decreased at a more or less uniform rate towards the top of the tree, whereas in tree 4 it fluctuated from bolt to bolt, reaching a maximum in bolt *c*, decreasing considerably in bolt *d*, and again increasing through bolts *e* and *f*.

In trees 1, 2, and 7 the specific gravity increased from the pith to the bark, whereas in trees 3, 4, and 6 the increase extended over only the first 2 to 3 inches after which there was a decrease towards the periphery. This condition was due to the very slow growth of the latter trees. In tree 3, however, this relation changed. The first samples around the pith had the greatest density in the lower bolts, whereas the intermediate or outer specimens were the heaviest in bolt *h*. In tree 5 the specific gravity increased from the pith to a maximum in the outer part of the heartwood and decreased from this point towards the bark.

The maximum and minimum specific gravity for the bolt rarely occurred on the same side of the tree. In trees 1, 2, 3, and 4 the greatest density was usually found in the closer-ringed material, whereas in tree 5 it was found in the medium- to broad-ringed material. In the latter case, however, the narrow-ringed material was found on the outer portion of the tree, and it is indicated that in general the tracheid length and density reached a maximum at about the same time (between 40 and 100 years), which varied somewhat with the growth rate of the tree. The material from the first 30 to 40 rings and the outer sapwood of the older trees was lighter in all cases than the material laid down during the 40- to 100-year period. It is evident that the rate of growth and age of the tree are the controlling factors determining the position of greatest density in the cross-section of a given tree.

Structural Properties

RATE OF GROWTH

By observing the relative number of growth rings per inch it was found that the material represented in this study divided itself into three natural divisions: trees 1, 2, and 7 *(Pinus Taeda)* of very rapid growth, tree 5 *(Pinus Taeda)* and tree 3 *(Pinus echinata)* of medium growth, and trees 4 and 6 *(Pinus palustris)* of very slow growth (table I).

Trees 1, 2, and 7 grew more than three times as fast as trees 4 and 6 and twice as fast as tree 3. Therefore, the first test

TABLE I

FIELD NOTES FOR THE SEVEN TREES STUDIED

	Tree 1 *P. Taeda*	Tree 2 *P. Taeda*	Tree 5 *P. Taeda*	Tree 7 *P. Taeda*	Tree 3 *P. echinata*	Tree 4 *P. palustris*	Tree 6 *P. palustris*
Where grown	Near Tylertown, Miss.	Near Tylertown, Miss.	About 8 mi. west of Sandy Hook, Miss., near La. border	In Tylertown, Miss.	Near Tylertown, Miss.	Near Tylertown, Miss.	Near Tylertown, Miss.
Approximate elevation	225 ft.	250 ft.	225 ft.	———	265 ft.	300 ft.	———
Soil conditions	Moderately dry clay loam	Set clay loam	Moist sandy clay	Sandy clay top soil, red clay sub-soil	Dry sandy clay	Dry sandy clay	Sandy clay top soil, red clay sub-soil
Exposure	West slope	Flat	Flat	Gentle south slope	Steep northwest slope	Steep southwest slope	Gentle north slope
Stand of timber	Dense second growth in old field	Moderately dense original swamp growth	Dense original hard wood with scattered pine trees	Open second growth hard and soft wood	Moderately dense original growth recently cut over, leaving open stand	Dense original growth recently cut over	Open second growth recently cut over
Height of tree	93 ft.	85 ft.	135 ft.	98 ft.	75 ft.	75 ft.	82 ft.
Diameter under bark at butt	18 in. (7 in. from ground)	18½ in. (30 in. from ground)	43 in.	19½ in.	17¾ in. (10 in. from ground)	16½ in. (10 in. from ground)	17½ in.
Approximate age	50 yrs.	50 yrs.	160 yrs.	60 yrs.	100 yrs.	160 yrs.	160 yrs.
Rate of growth	Rapid	Rapid	Medium to rapid except for very slow growing outer 4 or 5 in.	Rapid	Moderately slow	Very slow	Very slow
Date of cutting	Oct. 4, 1930	Oct. 4, 1930	Oct. 2, 1931	Feb. 1, 1933	Oct. 4, 1930	Oct. 4, 1930	Dec. 6, 1932

specimens near the pith of trees 1 and 2 represented only three to four years' growth, those of tree 5, six to fourteen, while there were more rings in some of the first specimens of tree 4 and especially tree 6 than there were in the entire cross-sections of trees 1, 2, and 7 (pl. 9). Trees 4 and 6 had from 10 to 44 rings per inch. This would give from 15 to 66 growth rings for each $1\frac{1}{2}$ x $1\frac{1}{2}$-in. specimen. Since the growth was not uniformly slow throughout the entire cross-section, there were few test specimens that contained more than 50 growth rings. The number of rings per inch is given in tables VII and VIII.

Trees 1, 2, 5, and 7 *(Pinus Taeda)* were more or less symmetrical in outline with the pith near the center, thus giving approximately the same number of test specimens on each side. Accordingly, there was little difference in the number of rings per inch at a given distance from the pith either on the opposite sides or at different levels.

Tree 3 *(Pinus echinata)* was slightly crooked and asymmetrical. The pith was nearer the bark on the south and west sides at the butt, whereas it was near the center of the log in bolt *h* at about 30 feet from the ground. The rings on the narrow side were necessarily closer, and since the pith was not straight it was impossible to cut an equal number of the specimens on each of the four sides. The growth was somewhat more rapid through the second and sometimes the third specimen in the lower bolts, and averaged about 8 rings per inch, but out near the bark, especially on the south and west sides, the growth decreased rapidly, giving about 15 to 20 rings per inch.

Trees 4 and 6 *(Pinus palustris)* were also crooked, with the pith off center. The *h* bolt in tree 4 and the *a* bolt in tree 6 were asymmetrical. Trees 4 and 6 had only two or three medium-width rings around the pith, after which the growth was extremely slow in the first 2 to 3 inches, except for a very few rings which occurred in scattered groups. The growth was somewhat more rapid in the outer part of the heartwood and in the sapwood towards the top of the tree.

These trees were characterized by periods of alternately

slow and more rapid growth. While the entire tree would be considered to have slow growth, the rate changed at intervals from about 20 rings per inch to more than 40 rings and back to 15 or 20 without following any particular pattern. Moreover, the width of ring varied greatly on opposite sides of the tree. Therefore, pieces taken at a given distance from the pith on opposite sides were not always matched specimens; one specimen may have been formed as much as forty years after the other. The broader rings often contained a certain amount of compression wood.

SUMMER WOOD

The percentage of summer wood was estimated macroscopically when the specimens were measured for testing. In the samples in which the resin ducts and shape of fiber were studied, the summer wood was measured under the microscope. Since in general the estimated values were found to be either too high, as in the specimens containing more than 50 per cent summer wood, or too low, only the percentage of summer wood obtained by microscopic measurements are reported here.

The first 2 to 5 rings of the tree usually contained very little summer wood. There was no sharp demarcation between early and late wood, and it was not uncommon for the first ring to be entirely without thick-walled cells such as were found in the summer wood farther from the pith. One was compelled to set an arbitrary limit on the spring and summer wood in such cases, since the cells were progressively smaller and thicker-walled towards the outer part of the ring. In some trees poor differentiation occasionally occurred in annual rings far from the pith. This was particularly noticeable in trees 1 and 2 *(Pinus Taeda)* where a large percentage of compression wood was found.

In the young rapid-growth trees 1, 2, and 7 *(Pinus Taeda)*, the percentage of summer wood increased from the pith to the periphery whereas in the older and slower-growing trees 3 *(Pinus echinata)*, 4 and 6 *(Pinus palustris)*, and 5 *(Pinus Taeda)* it reached a maximum some distance from the bark and then decreased. (Mohr and Roth, '97, found this to be true of

all old trees.) In the close-ringed trees a maximum of summer wood was often reached within the first 2 to 3 inches of the pith, with little variation over the rest of the cross-section.

The percentage of summer wood varied a great deal with height in the tree. Trees 1 and 2, representing vigorous-growth *Pinus Taeda,* averaged nearly twice as much summer wood at the butt as at a point 30 feet higher. In the slower-growing trees 3 *(Pinus echinata)* and 6 *(Pinus palustris),* the decrease was not so rapid, and in tree 4 *(Pinus palustris),* the percentage of summer wood decreased progressively but not uniformly from butt to top.

The "average" percentage of summer wood may be misleading in rapid-growth trees, since in a given cross-section considerable areas near the pith had as little as 15 per cent summer wood whereas only a few inches farther out there was often 60 per cent or more. Furthermore, the density of summer wood varied in the individual trees and particularly in trees of different species, as will be discussed in detail later.

SIZE AND DISTRIBUTION OF THE RESIN DUCTS

Resin ducts are canal-like intercellular spaces surrounded by parenchyma cells. The cells immediately around them, known as epithelial cells, were thin-walled and were more or less equilateral. The succeeding cells radiating from the resin ducts had increasingly thicker walls and became elongated until they blended into the tracheids. It was not uncommon for septate tracheids to occur in this transition zone.

Table II shows the size and distribution of the resin ducts both as to height and to distance from the pith in the tree. The samples for study were taken from the specimens used for the compression tests.

There was no attempt to determine the lengths of the longitudinal resin ducts which were distributed throughout the xylem. They were more abundant immediately around the pith, especially in the first 5-10 annual rings. The first ring often contained as many as 500 resin ducts, while farther out towards the periphery of the stem they were most numerous in the regions of transition from spring to summer wood and

TABLE II

THE SIZE AND DISTRIBUTION OF THE RESIN DUCTS AND THE PERCENTAGE OF AREA OCCUPIED BY THEM. THE PERCENTAGE OF SUMMER WOOD IS GIVEN IN TABLES VII AND VIII

Specimen	Number of resin ducts per sq. cm.		Mean diameter in mm.	Percentage of area of cross-section
	spring wood	summer wood		
Tree 1—*Pinus Taeda* (loblolly pine)				
1aN2	33	64	0.20	1.32
1aN4	6	79	0.22	1.61
1aN6	1	58	0.23	1.68
1aN7	2	48		1.62
1aN8	2	54	0.24	1.89
1dN2	43	52	0.19	1.25
1dN4	2	70	0.21	1.10
1dN6	0	55	0.23	1.05
1hN2	34	86	0.20	1.26
1hN4	41	63	0.22	1.82
1hN6	6	72	0.23	1.24
Tree 2—*Pinus Taeda* (loblolly pine)				
2aN2	54	71	0.20	1.86
2aN4	34	70	0.22	2.08
2aN5	25	50		1.65
2aN6	15	42	0.23	1.32
2aN8	8	47	0.24	1.47
2dN1	54	85		2.05
2dN2	38	57	0.21	1.51
2dN4	26	51	0.23	1.58
2dN5	28	45		1.61
2dN6	23	39	0.24	1.40
2dN8	17	43	0.25	1.47
2hN1	69	85		2.50
2hN2	34	58	0.21	1.34
2hN3	35	72		1.85
2hN4	38	60	0.23	1.85
2hN5	36	58		1.95
2hN6	35	43	0.24	1.71
2hN8	27	49	0.24	1.70
Tree 5—*Pinus Taeda* (loblolly pine)				
5bN1	45	55	0.19	1.53
5bN3	53	21	0.20	1.13
5bN5	53	20	0.21	1.13
5bN7	50	37	0.23	1.74
5bN9	46	43	0.23	1.83
5bN11	40	29	0.23	1.37
5bN13	8	48	0.22	1.23
5bN15	17	54	0.21	1.27

TABLE II (Continued)

Specimen	Number of resin ducts per sq. cm.		Mean diameter in mm.	Percentage of area of cross-section
	spring wood	summer wood		
Tree 5 (Continued)				
5iN1	40	72	0.19	1.31
5iN3	29	42	0.21	1.14
5iN5	15	52	0.22	1.17
5iN7	29	28	0.23	1.19
5iN9	20	43	0.23	1.22
5iN11	9	39	0.23	0.92
5iN13	17	95	0.22	1.67
5qN1	40	52	0.19	1.19
5qN3	33	37	0.20	1.18
5qN5	27	46	0.21	1.12
5qN7	23	57	0.23	1.42
5qN9	9	75	0.23	1.25
5qN11	17	106	0.22	1.61
Tree 7—*Pinus Taeda* (loblolly pine)				
7aN1	17	76	0.20	1.12
7aN3	21	50	0.22	1.20
7aN5	21	34	0.25	1.29
7aN7	13	38	0.25	1.25
7gN1	21	44	0.21	1.03
7gN3	20	41	0.22	1.07
7gN5	11	48	0.23	1.12
7gS7	16	52	0.23	1.31
7mN1	23	35	0.21	0.92
7mN3	9	58	0.23	1.18
7mN5	11	51	0.24	1.37
7mS7	16	50	0.23	1.29
Tree 3—*Pinus echinata* (shortleaf pine)				
3aN1	18	30	0.19	0.69
3aN2	38	48		1.24
3aN3	23	19	0.20	0.66
3aN4	24	22		0.72
3aN5	33	26	0.20	0.91
3aN6	15	28		0.73
3aN7	30	28	0.21	1.00
3aN8	19	22		0.71
3aN9	30	26	0.21	1.00
3aN10	30	26		0.97
3dN1	24	43	0.19	0.89
3dN3	23	22	0.20	0.71
3dN5	29	30	0.20	0.92
3dN7	19	21	0.21	0.69

TABLE II (Continued)

Specimen	Number of resin ducts per sq. cm.		Mean diameter in mm.	Percentage of area of cross-section
	spring wood	summer wood		
Tree 3 (Continued)				
3hN1	22	32	0.19	0.70
3hN2	22	49		0.89
3hN3	15	20	0.20	0.52
3hN5	20	15	0.20	0.57
Tree 4—*Pinus palustris* (longleaf pine)				
4aN2	19	67	0.19	1.07
4aN4	27	45	0.20	1.10
4aN6	27	45	0.21	1.21
4aN8	37	39	0.22	1.34
4cN1	27	67	0.20	1.08
4cN2	35	69		1.22
4cN3	27	63	0.21	1.27
4cN5	36	34	0.22	1.17
4hN1	33	23	0.19	1.15
4hN2	25	44		0.85
4hN3	28	49	0.22	1.06
4hN4	26	47		0.96
Tree 6—*Pinus palustris* (longleaf pine)				
6aN1	18	78	0.20	1.45
6aN3	14	31	0.23	0.90
6aN5	29	50	0.25	1.82
6aS7	22	41	0.25	1.41
6eN1	43	92	0.20	1.69
6eN3	27	62	0.22	1.51
6eN5	20	56	0.23	1.32
6iN1	38	53	0.20	1.32
6iN3	24	45	0.22	1.07
6iN5	33	47	0.24	1.65

in the summer wood, with very few in the first part of the spring wood. This was particularly noticeable in the broad-ringed material (pl. 10, fig. 1). Resin ducts were often aggregated in false rings and occasionally formed a definite band near the middle of the spring wood rings. In narrow-ringed material the resin ducts were scattered promiscuously

throughout the annual ring, but, with the exception of certain specimens in *Pinus echinata,* they were more numerous in the summer-wood portion.

The mean diameter of the resin ducts increased from the pith towards the periphery of the stem, reaching a maximum near the outer sap-wood, but occasionally it decreased slightly in the last-formed rings in the older trees. The greater number of resin ducts in the first and last rings was quite marked in tree 5.

The percentage of cross-sectional area taken up by resin ducts was slightly greater in the rapid-growth *Pinus Taeda* than in the other species. In *Pinus Taeda* the area taken up by them increased in the summer-wood portion of the ring towards the periphery of the stem and decreased markedly in the spring wood in the same direction, whereas in *Pinus palustris* and in *Pinus echinata* there was no consistent change. In *Pinus echinata* the resin ducts were smaller and fewer in number than in the other species. On the whole there was less than 1 per cent of the cross-sectional area taken up by them. In the summer wood of *Pinus palustris* and *Pinus Taeda,* however, it was not uncommon for more than 2 per cent, and for small areas as much as 3 per cent, of the area to be occupied by resin ducts.

In general, the mean diameter of the resin ducts was slightly greater in the spring wood than in the summer wood, although the diameters in the tangential direction were frequently greater in the summer wood, where the resin ducts were often aggregated in rows of from 2 to 5 (pl. 10, fig. 2). This was particularly true of *Pinus Taeda,* where the aggregations were occasionally about 1 mm. in width. Such groups also occurred in the spring wood but they were much less common. In no instance was there any evidence of injury in the vicinity of these groups of resin ducts.

There was no consistent relationship between the number of resin ducts and the height of the tree. However, as a whole the mean diameters of the resin ducts, and thus the area taken up by them, were greatest in the lower bolts. They decreased slightly through the middle portion of the tree and usually in-

creased again near the top. This condition, however, was often reversed in individual specimens from the outer portion of the tree.

Areas of parenchyma cells occurred, other than those around the resin ducts, which areas contained secretory or resin cells. The greater number of them were thick-walled. These areas of parenchyma cells were more common in the spring wood and occurred in all three species.

SIZE AND DISTRIBUTION OF THE WOOD RAYS

The significance of the wood rays in relation to the structure and strength of wood attracted the attention of Nördlinger ('60), and in 1881 he published his findings on the number of rays per mm. for a number of hard woods and noted the numerous small rays in the conifers.

Essner ('82) studied the distribution and height of the rays in various species of conifers. He found little or no difference in the number of rays on the narrow side of trees caused by unequal growth rate, but did observe that certain specimens of *Cupressus sempervirens* of symmetrical growth had 83 rays per mm. on one side as compared with 62 on the opposite side. The height of the rays increased from the pith to the bark.

Jaccard ('15) studied the rays at the various levels in the tree and in the branches of *Sequoia* and *Picea.* In the main stem or trunk the rays decreased in number from a maximum at the base to a minimum within the lower part of the trunk and again increased towards the top of the tree. They were more numerous but smaller in the branches where the greatest number was found in the areas of compression wood on the lower side. He observed that as the rays decreased in number the tracheids increased in size. Myer ('22) reported the ray volumes of a large number of American woods and pointed out that the hard woods had a much greater ray volume than the conifers, an average of 17.04 and 7.08 per cent, respectively. The ray volumes for the southern pines were 7.63 per cent for *Pinus Taeda,* 8.05 per cent for *Pinus echinata,* and 8.30 per cent for *Pinus palustris.* In summarizing his results, Myer ('30) stated that the ray volume of white pine, hemlock,

and sugar maple was greatest at the top of the merchantable log and second greatest at the stump level, while a reduction was found at the 16-ft. level. His observations as recorded in the paper, however, show the maximum at the stump level. If the latter is the correct conclusion, it agrees with other work. There was no consistent relationship between the ray volume and the different sites from which the trees were cut. The ray height in the sugar maple was least at the stump level but its width was greatest at this point. Myer's ('30) observations on the effects of the site do not agree entirely with those of Hartig ('01) on the oak. The latter found a greater ray volume in the plants grown in the open and an increase in ray volume if shaded plants were suddenly given full illumination. Forsaith ('20) found a reduction of rays in certain alpine forms, and Shope ('27) found that the size of the ray cells, as well as other cells, increased in the aspen at higher elevations. Harlow ('27) was unable to find any consistent variations in the ray volume of plants from different sites. He observed, however, that it was greater at the base of the tree. There was a great variation in the size, type, and distribution of rays in the different species of hard woods, depending upon their ages, according to Zache ('86), Hartig ('94), and Eames ('10).

It is evident that there is not sufficient data to draw any reliable conclusions as to the size and distribution of wood rays due to environmental conditions or their effects on strength, but it is universally agreed that the ray volume of conifers is greatest in the butt of the tree and least in the main trunk at a height of 20 to 40 feet.

In this investigation the wood rays of southern pine were found to be largely of the linear (uniseriate) and fusiform (multiseriate) types. The latter contained solitary lateral resin ducts. Occasional biseriate rays were observed, but these were rare and of little significance in this study. The area of the rays was determined by taking two-thirds of the area of a circumscribed parallelogram based upon the mean height and breadth of the rays in a given specimen.

The study was made on tangential sections mounted in Canada balsam, and the measurements were made by means of a

micrometer eyepiece. The number per mm. was determined by taking the average from 100 to 400 sq. mm. This was made possible by using a cover glass on which fine lines were etched at a distance of about 0.8 mm. The number of rays between the lines could be accurately counted for the entire area of a large section, since the lines were sufficiently transparent to allow the counting of rays beneath them.

Fusiform Rays.—The width (lateral diameter), the height (longitudinal diameter), the distribution of the fusiform rays, and the percentage of area occupied by them is given in table III. The width of these rays varied from about 0.05 mm. to about 0.08 mm. They were smaller near the pith and reached a maximum near the periphery of the stem. There was little difference in the breadth of the rays in the different species. The height of the fusiform rays, in most specimens, tended to decrease from the butt of the tree towards the top, reaching a minimum through the middle portion of the tree. In tree 7 *(Pinus Taeda),* however, it increased slightly from the base to the top.

The fusiform rays were more or less constant in number at the different heights in the tree, but they decreased very markedly from the pith towards the periphery of the stem at all levels studied. However, since the number per unit area often varied more than 100 per cent in local areas, it was necessary to study a number of sq. cm. in order to get a reliable average. In 1aN6 (table III) there was an average of 83 fusiform rays per sq. cm., and in certain sections taken at this point there were more than 100 per sq. cm.

Fusiform rays were more abundant in *Pinus Taeda* than in either *Pinus palustris* or *Pinus echinata,* but there was no pronounced difference in the height of these rays in either species. There was no indication of a fixed pattern or sequence of occurrence in any of the species. They were scattered throughout, but occurred occasionally in groups.

Linear Rays.—The linear rays were very numerous in the first growth ring, where there were from 70 to 120 minute rays per sq. mm. immediately adjacent to the pith. They were

TABLE III

THE SIZE AND DISTRIBUTION OF THE FUSIFORM RAYS

Specimen	Approximate number of rings from the pith	Number of rays per sq. cm.*	Mean width in mm.	Mean height in mm.	Percentage of area occupied by fusiform rays
Tree 1—*Pinus Taeda* (loblolly pine)					
1aN2	3	71	0.061	0.343	0.99
1aN4	16	50	0.063	0.436	1.02
1aN6	34	83	0.068	0.428	1.61
1aN8	45	38	0.075	0.521	0.99
Ave.		60	0.066	0.432	1.15
1dN2	3	76	0.060	0.350	1.25
1dN4	13	50	0.059	0.422	0.84
1dN6	30	47	0.062	0.422	0.82
1dN8	40	45	0.073	0.504	1.10
Ave.		55	0.063	0.425	1.00
1hN2	3	43	0.062	0.359	0.64
1hN4	11	38	0.067	0.398	0.72
1hN6	25	38	0.070	0.500	0.89
Ave.		40	0.066	0.419	0.75
Tree 2—*Pinus Taeda* (loblolly pine)					
2aN2	3	57	0.060	0.328	0.75
2aN4	10	38	0.063	0.462	0.73
2aN6	20	30	0.072	0.537	0.78
2aN8	35	31	0.066	0.514	0.70
Ave.		39	0.065	0.460	0.74
2dN2	3	56	0.078	0.393	0.77
2dN4	10	35	0.055	0.410	0.54
2dN6	19	39	0.058	0.400	0.60
2dN8	30	31	0.066	0.500	0.67
Ave.		40	0.062	0.427	0.65
2hN2	3	59	0.055	0.250	0.76
2hN4	10	38	0.067	0.379	0.65
2hN6	17	35	0.063	0.470	0.73
2hN8	28	30	0.067	0.484	0.65
Ave.		41	0.063	0.396	0.70

* Note that the number of fusiform rays is given in sq. cm.

TABLE III (Continued)

Specimen	Approximate number of rings from the pith	Number of rays per sq. cm.*	Mean width in mm.	Mean height in mm.	Percentage of area occupied by fusiform rays
Tree 5—*Pinus Taeda* (loblolly pine)					
5bN1	10	54	0.063	0.328	0.75
5bN7	46	37	0.076	0.487	0.91
5bN1-5	125	26	0.075	0.744	0.97
Ave.		39	0.071	0.519	0.87
5iN1	6	67	0.065	0.312	0.91
5iN7	40	34	0.077	0.434	0.74
5iN13	105	28	0.069	0.577	0.74
Ave.		43	0.070	0.441	0.79
5qN1	4	58	0.057	0.288	0.63
5qN5	27	39	0.068	0.488	0.86
5qN11	88	34	0.073	0.522	0.86
Ave.		44	0.066	0.433	0.78
Tree 7—*Pinus Taeda* (loblolly pine)					
7aN1	5	56	0.059	0.330	0.73
7aN3	15	43	0.065	0.368	0.69
7aN5	26	37	0.084	0.373	0.77
7aN7	40	35	0.085	0.402	0.79
7aN9	50	34	0.067	0.414	0.63
Ave.		41	0.072	0.377	0.72
7gN1	4	46	0.056	0.367	0.63
7gN3	13	42	0.061	0.372	0.63
7gN5	25	38	0.063	0.371	0.59
7gN7	39	38	0.058	0.410	0.60
Ave.		41	0.059	0.380	0.61
7mN1	4	62	0.064	0.321	0.85
7mN3	13	50	0.066	0.423	0.93
7mN5	26	43	0.067	0.459	0.88
7mN7	40	45	0.066	0.485	0.89
Ave.		50	0.066	0.422	0.89

* Note that the number of fusiform rays is given in sq. cm.

TABLE III (Continued)

Specimen	Approximate number of rings from the pith	Number of rays per sq. cm.*	Mean width in mm.	Mean height in mm.	Percentage of area occupied by fusiform rays
Tree 3—*Pinus echinata* (shortleaf pine)					
3aN1	12	32	0.057	0.474	0.57
3aN3	35	43	0.059	0.485	0.82
3aN5	59	33	0.061	0.497	0.67
3aN7	84	32	0.065	0.532	0.74
3aN9	108	28	0.068	0.550	0.70
Ave.		36	0.062	0.507	0.70
3dN1	16	30	0.057	0.400	0.46
3dN3	44	28	0.059	0.430	0.47
3dN5	70	28	0.063	0.482	0.57
3dN7	98	27	0.068	0.504	0.61
Ave.		28	0.062	0.454	0.53
3hN1	12	44	0.057	0.370	0.63
3hN3	35	28	0.063	0.389	0.46
3hN5	61	27	0.068	0.410	0.50
Ave.		33	0.062	0.389	0.53
Tree 4—*Pinus palustris* (longleaf pine)					
4aN2	36	43	0.054	0.335	0.52
4aN6	122	31	0.063	0.474	0.67
4aN8	150	31	0.071	0.614	0.90
Ave.		36	0.062	0.474	0.66
4dN1	40	39	0.061	0.441	0.71
4dN3	103	33	0.068	0.497	0.74
4dN5	145	28	0.073	0.543	0.69
Ave.		33	0.067	0.493	0.71
4hN1	21	36	0.068	0.368	0.50
4hN3	67	30	0.071	0.428	0.61
4hN5	110	30	0.075	0.489	0.73
Ave.		32	0.071	0.425	0.65

* Note that the number of fusiform rays is given in sq. cm.

TABLE III (Continued)

Specimen	Approximate number of rings from the pith	Number of rays per sq. cm.*	Mean width in mm.	Mean height in mm.	Percentage of area occupied by fusiform rays
Tree 6—*Pinus palustris* (longleaf pine)					
6aN1	43	31	0.056	0.372	0.35
6aN3	67	26	0.066	0.389	0.48
6aN5	106	27	0.070	0.480	0.62
6aN7	142	28	0.072	0.480	0.65
Ave.		28	0.066	0.430	0.52
6eN1	25	28	0.053	0.348	0.34
6eN3	75	33	0.057	0.427	0.52
6eN5	115	25	0.069	0.406	0.46
Ave.		29	0.060	0.394	0.44
6iN1	28	30	0.059	0.342	0.42
6iN3	72	28	0.071	0.415	0.57
6iN5	112	28	0.066	0.474	0.61
Ave.		29	0.065	0.410	0.53

* Note that the number of fusiform rays is given in sq. cm.

naturally very small at this point, being only about 0.08 mm. or less in height. As the stem increased in diameter the rays were separated more and more, new ones originating with the additional tiers of tracheids. Since the number of rays did not increase so rapidly as the diameter of the stem, there was only about one-fourth to one-sixth as many rays per sq. mm. in the outer portion as near the pith.

The decrease in number of rays was not uniform in all specimens nor all portions of a given specimen. At certain areas in the tree marked only by a sudden change in growth rate, there was a temporary increase in number of rays which was followed by the usual rate of decline.

Table IV gives the number of linear rays per sq. mm., their mean heights and breadths, and the approximate area taken up by them in the different portions of the tree as indicated. Since the size of the rays often increased more rapidly than the number decreased, in many bolts the area occupied by them

was greatest in the outer portion of the tree. This was true of all three species studied.

The breadth of the rays increased centrifugally only slightly, from about 0.022 to 0.028 mm. The height increased more or less rapidly, attaining an average of more than 0.2 mm. in certain trees, although individual rays were occasionally 1 mm. or more in height in *Pinus Taeda.*

The number of rays was practically constant for a given area at different heights in the tree, except in the butt where there were a few more. Their breadth was also reasonably uniform throughout the tree, but the height decreased slightly from the stump level through the middle portion of the trunk, with a second maximum in the region of the crown. For this reason the area taken up by the rays was greatest at the base and least at about the 30- to 40-ft. level.

The linear rays, like the fusiform, were more numerous at a given distance from the pith and slightly larger in the specimens of *Pinus Taeda* than in either *Pinus palustris* or *Pinus echinata,* the latter of which had fewer and smaller rays. If, however, the age of the tree were taken into consideration, *Pinus echinata* and *Pinus palustris* had a greater number of rays than did *Pinus Taeda* due to the narrow rings near the pith.

Tables VII and VIII show that *Pinus echinata* had a lower percentage of area occupied by resin ducts and wood rays than *Pinus palustris,* and that *Pinus Taeda* had a greater percentage than the other two. It was unfortunate that there was only one tree of *Pinus echinata* available for study, since there were certain variations shown in the different trees of the other two species. Furthermore, tree 3 grew on a north slope where it was probably shaded a good part of the time, which, according to Hartig's ('01) observations, would account for the smaller number of rays.

There were only slight variations in number or size of linear rays on opposite sides of a given tree, but areas containing a large amount of compression wood had somewhat broader rays and a greater number of both fusiform and linear rays, which increased the ray volume somewhat.

TABLE IV

THE SIZE AND DISTRIBUTION OF THE LINEAR RAYS

Specimen	Appoximate number of rings from the pith	Number of rays per sq. mm.	Mean width in mm.	Mean height in mm.	Percentage of area occupied by linear rays
Tree 1—*Pinus Taeda* (loblolly pine)					
1aN1	3	32	0.023	0.161	7.93
1aN3	16	29	0.024	0.168	7.80
1aN5	34	28	0.025	0.182	7.80
1aN7	45	28	0.026	0.191	9.26
1dN1	3	30	0.022	0.172	8.10
1dN3	13	25	0.023	0.174	7.10
1dN5	30	24	0.025	0.186	7.50
1dN7	40	22	0.026	0.190	7.50
1hN1	3	26	0.023	0.180	7.35
1hN3	11	24	0.026	0.182	7.13
1hN5	25	22	0.026	0.190	7.14
Tree 2—*Pinus Taeda* (loblolly pine)					
2aN1	3	30	0.024	0.167	8.00
2aN3	10	27	0.026	0.170	7.93
2aN5	20	26	0.026	0.204	9.20
2aN7	35	25	0.027	0.194	8.72
2dN1	3	29	0.024	0.159	7.25
2dN3	10	27	0.025	0.165	7.27
2dN5	19	26	0.026	0.184	8.05
2dN7	30	25	0.027	0.198	8.92
2hN1	3	30	0.025	0.161	7.60
2hN3	10	27	0.026	0.164	7.23
2hN5	17	25	0.026	0.169	7.12
2hN7	28	24	0.027	0.207	9.10
Tree 5—*Pinus Taeda* (loblolly pine)					
5bN1	10	28	0.022	0.160	6.15
5bN7	46	24	0.025	0.186	7.60
5bN15	125	20	0.025	0.244	8.75
5iN1	6	26	0.022	0.150	5.43
5iN7	40	23	0.024	0.170	7.02
5iN13	105	20	0.026	0.194	7.00
5qN1	4	25	0.023	0.160	5.55
5qN5	27	23	0.026	0.200	9.74
5qN11	88	22	0.025	0.198	7.20

TABLE IV (Continued)

Specimen	Appoximate number of rings from the pith	Number of rays per sq. mm.	Mean width in mm.	Mean height in mm.	Percentage of area occupied by linear rays
Tree 7—*Pinus Taeda* (loblolly pine)					
7aN1	5	31	0.023	0.155	7.25
7aN3	15	30	0.023	0.168	8.37
7aN5	26	29	0.024	0.171	8.17
7aN7	40	25	0.024	0.173	7.02
7aN9	56	26	0.024	0.162	6.90
7gN1	4	30	0.023	0.141	6.46
7gN3	13	27	0.023	0.158	6.45
7gN5	25	26	0.024	0.153	6.54
7gN7	39	28	0.024	0.127	5.74
7mN1	4	30	0.023	0.149	6.65
7mN3	13	28	0.024	0.151	6.67
7mN5	26	27	0.024	0.150	6.55
7mN7	40	29	0.024	0.151	7.15
Tree 3—*Pinus echinata* (shortleaf pine)					
3aN1	12	25	0.023	0.145	5.30
3aN3	35	24	0.023	0.148	5.44
3aN5	59	23	0.023	0.153	5.37
3aN7	84	22	0.024	0.160	5.63
3aN9	108	21	0.025	0.166	5.80
3dN1	16	25	0.022	0.140	5.14
3dN3	44	24	0.023	0.145	5.35
3dN5	70	23	0.024	0.157	5.76
3dN7	98	22	0.024	0.160	5.62
3hN1	12	26	0.022	0.143	5.45
3hN3	35	24	0.023	0.149	5.50
3hN5	61	21	0.024	0.158	5.31
Tree 4—*Pinus palustris* (longleaf pine)					
4aN2	36	24	0.023	0.151	5.94
4aN6	122	24	0.024	0.159	6.50
4aN8	150	23	0.026	0.167	7.05
4dN1	40	25	0.024	0.152	5.95
4dN3	103	24	0.024	0.154	6.04
4dN5	145	23	0.026	0.158	6.11
4hN1	21	24	0.023	0.148	5.30
4hN3	67	24	0.024	0.151	5.47
4hN5	110	23	0.024	0.152	5.60

TABLE IV (Continued)

Specimen	Appoximate number of rings from the pith	Number of rays per sq. mm.	Mean width in mm.	Mean height in mm.	Percentage of area occupied by linear rays
Tree 6—*Pinus palustris* (longleaf pine)					
6aN1	43	28	0.022	0.141	5.79
6aN3	67	25	0.023	0.154	5.90
6aN5	106	23	0.024	0.208	7.64
6aN7	142	23	0.025	0.195	7.46
6eN1	25	27	0.021	0.145	5.45
6eN3	75	24	0.022	0.145	5.18
6eN5	115	23	0.024	0.168	6.50
6iN1	28	25	0.021	0.143	5.61
6iN3	72	24	0.023	0.159	5.76
6iN5	112	23	0.024	0.202	7.80

TRACHEID DIMENSIONS

The length of the tracheids in coniferous wood varies with the age of the tree at the time the cells are formed. Sanio ('72) found that in *Pinus sylvestris* the tracheids increased in length from the first ring to a maximum in the 45th ring, after which they remained constant. This led him to believe that the size of the cell had a fixed relationship to the age of the tree, but this has been proved untrue. Mell ('10) observed that the wood fibers were longest, for any given annual ring, in rapid-growth trees. Bailey and Shepard ('15) noted that in certain species of conifers the length of the tracheids increased from the inner portion of the secondary xylem up to a certain age, after which it fluctuated. No constant length of tracheids was found. In addition, Gerry ('15) found that the fiber length increased from the butt towards the crown for about two-thirds of the height of the tree. The longest fibers in a given ring were in the first spring-wood cells and the shortest in the last summer-wood cells. Gerry ('16), contrary to Mell, was not able to find any correlation between fiber length and rate of growth in Douglas fir, but noted a rapid increase in all dimensions of the fiber in the first twenty years of growth. Lee and Smith ('16), on the other hand, found that in Douglas fir the

longest fibers in a given ring were in the summer wood. Further study on the fiber length of different species was made by Bailey and Tupper ('18), Kribs ('28), and Gerry ('29). MacMillan ('25) found that there was a greater percentage of short fiber in red spruce grown under suppressed conditions than in that grown in free, which agrees with the findings of Mell.

In this study, the tracheids were prepared for measuring by first macerating them (Jeffrey method) and then teasing them apart on the stage of a compound microscope. Measurements were made by means of a micrometer eye-piece. Each figure shown in tables VII and VIII is the average of 200–500 tracheids.

The tracheids were very short in the first ring around the pith, with an average length of 1.5 mm. or less. Their length increased rapidly through the first 5 to 10 rings and more gradually for the remainder of the growth of the tree or at least until the tree reached the age of 100 years or more. No maximum length was determined in this material.

In a given tree the length of the tracheids was dependent upon the number of years of growth and not upon the distance from the pith; therefore, in broad-ringed material they were shorter at a given distance from the pith than in the narrow-ringed material. This was very outstanding in the different trees used in this study. In trees 1, 2, and 7, and somewhat in tree 5 (all *Pinus Taeda*), the rings were broad near the pith and for some distance out, whereas in tree 3 *(Pinus echinata)* and particularly in trees 4 and 6 *(Pinus palustris)* the rings were very narrow. Consequently, the average length of the tracheids in the first test specimen near the pith in trees 3, 4, and 6 was as great as it was in the second or third specimen from the pith in trees 1, 2, 5, and 7. The tracheids representing approximately the same year's growth were slightly longer in the rapid-growth *Pinus Taeda* than in the other species.

At the successively higher levels in the tree, the tracheids increased in length more rapidly from the pith to the bark. The result was that the average length for the cross-section tended to increase towards the top of the tree. The tracheids in a given ring increased in length towards the top, especially for the first few feet from the butt.

Groups of short tracheids were often found throughout the cross-section of the tree, particularly in the spring wood which usually contained the longest ones. Where the short tracheids occurred, there was frequently a difference of as much as 100 per cent in their lengths. Therefore, the average length of the summer wood tracheids was usually greater than that of the spring wood. Crooked tracheids and areas of compression wood usually had a greater variation in length than material containing straight tracheids with concentric laminae.

In trees 1, 2, and 7 *(Pinus Taeda)*, which were of about the same age and growth rate, the tracheid length was approximately the same for a given position. The average length of the tracheids for the various levels represented by the specimens is given in tables VII and VIII. The slight differences found on the opposite sides of the tree were insignificant. In tree 5 *(Pinus Taeda)* the somewhat narrower rings were correlated with a more rapid increase in length of tracheids from pith to periphery, especially in bolts *i* and *q*. In this tree the average length was 5 mm. or more in the outer specimens except in bolt *b* which was from the butt log.

Tree 3 *(Pinus echinata)* had approximately the same length tracheids for a given growth ring as trees of *Pinus Taeda*, but the rings were much narrower; therefore, the average length in the first test specimens near the pith was approximately 4 mm. It is evident that these test specimens contained a large percentage of tracheids which were 4.5 mm. or more in length, since those of the first rings were very short.

Trees 4 and 6 *(Pinus palustris)* had somewhat shorter tracheids for a given ring than the *Pinus Taeda* trees studied, but the average length for a given specimen was greater due to the very slow growth.

The mean diameter of the tracheids increased with the increase in length. The diameter of the spring-wood tracheids was from one-third to one-half greater than those of the summer wood. The maximum diameters observed for the spring wood tracheids of *Pinus Taeda* were about 0.06 mm. in the first 2 or 3 rings to about 0.085 mm. in the outer part of the trees. For corresponding positions in the tree, *Pinus echinata*

showed an increase from about 0.06 to 0.072 mm., and *Pinus palustris* from 0.05 to 0.07 mm. In the summer wood of *Pinus Taeda* the maximum diameters increased from about 0.04 mm. in the first rings to about 0.052 mm. in the outer part of the trees, in *Pinus echinata* from about 0.04 to 0.049 mm., and in *Pinus palustris* from about 0.032 to 0.044 mm. The average diameter was somewhat lower in all cases and increased in about the same ratio, but since the tracheids tapered from near the middle towards either end, it was very difficult to determine the average diameters. To give a true value it would be necessary to isolate the cells and measure them at a given point but the large numbers necessary for a significant figure made this impractical. A cursory study showed that the average diameter of the tracheids increased from the base of the tree towards the top, at least for the first few feet. Since the wood rays and resin ducts vary within small limits, the thickness of the cell walls was best reflected in the specific gravity. Therefore, it was considered unnecessary to make a statistical study of them.

TRACHEID IRREGULARITIES

The length of the tracheids, as stated above, had a definite relation to their age and position in the tree, while the differences in general contour of the cells and the thickness of the cell walls were more or less correlated with the spring and summer wood. On the other hand, the tracheids varied greatly in degree of straightness with little relation to position in the tree. In general, they were straighter in the upper and outer portions, but this relation did not hold for all trees or for all positions in a given tree. Areas of crooked tracheids occurred in any portion of the tree, and were common in the butt bolts and the first rings around the pith. Crooked tracheids occurred characteristically in compression wood but also around knots, adventitious buds, leaf traces, injuries, and in the otherwise clear wood.

Crooked tracheids can usually be detected by the irregularity of the rupture when small slivers are split from the specimen, by a rough or splintery surface in dressed material

(especially in material of high density), and by a waviness in the annual rings or a curly grain. Crooked tracheids must not be confused with spiral-grain, which also may give a rough surface in dressed material, although they were found to be associated with it in tree 5. Cross- or spiral-grained material gives a rough surface if dressed against the grain and a smooth surface if dressed with it, whereas material containing unusually crooked tracheids gives an uneven surface when dressed in either direction.

Straight uniform tracheids, except for slight curvatures at the wood rays (pl. 10, fig. 3), with tapering sharp-pointed ends were found in all the trees studied, but they were accompanied by areas of crooked tracheids. Trees 1, 2, and 7 contained a large percentage of tracheids which were straight except for recurved or crooked ends. This was especially true of the very rapid-growth material near the pith and the slow-growth material in the sapwood of the older trees. In tree 5 and in certain areas of all the trees, the tracheids were wavy throughout (pl. 10, fig. 4). The V-shaped markings shown in pl. 9, fig. 3 (5bN12) were made up of these crooked tracheids. In general, where this irregularity was not due to a knot or other disturbance as stated above, the curve of one tier was opposite that of the adjacent tier so that the tracheids crossed each other (pl. 10, fig. 5). The intercellular spaces caused by the crossing of the cells prevented compactness of form and interrupted the gluing layer.

Other elements that might be classed as inferior in strength properties are compression wood, and the occurrence of parenchyma cells around the resin ducts (pl. 10, fig. 6) and in limited areas which appeared to be callus tissue. Compression wood will be discussed in detail in the next section.

THE STRUCTURE OF THE CELL WALL

The physical properties and chemical constituents of the cell walls of plants in relation to microscopic structure have attracted the attention of botanists for many years. Nägeli ('64) observed a definite concentric lamination of broad light bands alternating with narrow dark ones in the cell walls of

flax fibers. He advanced the theory of alternate layers of cell wall material each containing different amounts of water. By the use of swelling reagents he observed striations on the surfaces of the fibers which he designated as fibrils. He noted further that the fibrils in adjacent laminae ran in opposite directions, each lying in a spiral around the cell. Anderson ('27) expressed the belief that the laminae were not alternate layers of water-rich and water-poor cellulose, but that they represented the boundary lines of the layers of cellulose. He later ('28a) modified his conclusions, stating that the alternate layers were of different chemical constituents, and ('28b) he observed that in the epidermis of *Clivia nobilis* the cell wall was made up of alternating layers of cellulose and pectin. Anderson ('27) and Ritter ('28) were able to separate the laminae from each other.

The middle lamella of lignified tissues was believed to be composed largely of lignin by Ritter ('25), Harlow ('27), Candlin and Schryver ('28), Harlow and Wise ('28), and Scarth, Gibbs, and Spier ('30), none of whom denied the possibility of pectin being present. The recent work of Kerr and Bailey ('33) states that the middle lamella is a combination of lignin and pectin. The secondary thickening may be composed either of cellulose and lignin (Scarth, Gibbs, and Spier, '30) or of cellulose alone. It is likely, however, that the secondary wall contains a certain amount of pectin. This is indicated by the recent work of Farr and Eckerson ('33) on the cotton fiber and of Kerr and Bailey ('33) on woody cells.

The amplification of the micellar theory of Nägeli by the use of X-rays as reviewed by Clark ('30) and Thiessen ('32) has demonstrated the dimensions and orientation of the micells, which have been found to lie at various angles to the axes of the fibers. Cotton fibers which have the greatest tensile strength are those with the fibrils parallel with the axes (Farr and Clark, '32).

Spierer constructed a special lens used as an oil-immersion objective (Seifriz, '31). Seifriz ('31) and Thiessen ('32), using the Spierer lens, reported diffraction patterns of macromicells (aggregates of true micells similar in shape to the lat-

ter) in plant cell walls. Thiessen reported the same phenomenon in partially decayed wood and coal.

The writer has been unable to substantiate this interpretation by the use of the Spierer lens at magnifications up to 2700 diameters,[4] but the laminae have been clearly outlined, thus showing in detail the gross structure of the cell wall. The cross-sections[5] of ordinary cells showed the laminae to be in the form of concentric rings or cylinders (pl. 11, fig. 1). It will be observed that the middle lamella was not differentiated from the secondary thickening. The same structure was readily demonstrated with direct illumination (pl. 11, fig. 2). Longitudinal sections of similar cells showed the laminae to be arranged uniformly and parallel with each other (pl. 11, fig. 3). The lumina of the cells appeared as dark bands, and in no instance was there any trace of macro-micells in the diffraction patterns.

"Compression wood," so-called because it was thought to be confined to the under side of limbs and leaning stems of Gymnosperms (originally described as *Rothholz* by German investigators because of its reddish color), has been described as tracheids that have rounded corners and that are characterized by a thick, heavily lignified, and spirally striated inner layer of the secondary wall.

There have been a number of theories advanced to explain the development of this type of wood, which have been reviewed by Kienholz ('30). The fact that these cells were found in abundance on the compression side of the tree led investigators to believe that they were caused by the compression stresses existing on the cells during growth, but recent findings indicate that they may be caused by gravity or by injury to the growing cells.

Both the spring and summer wood of a growth ring, or only a portion of either, may be made up of compression wood. Thin layers of it occurred in the summer-wood portion of nar-

[4] In addition to the Spierer lens, which is an oil-immersion objective (90 x), a 30 x ocular was used.

[5] The sections were made both with and without desilicification by the use of hydrofluoric acid. The same structure was revealed in either case. This was also true of the sections of compression wood.

row rings, and isolated cells were found in both the spring and summer wood, thus completely hidden from the unaided eye (pl. 11, fig. 4). It could be readily recognized in stained sections under the microscope, however, by the difference in color caused by differential staining, the contour of the cells, and particularly by the structure of the secondary thickening of the cell walls. The white spots to be seen in the photograph cited above were due to checks which were clearly visible in the longitudinal section (pl. 11, fig. 5) and in the cross-section at higher magnification (pl. 11, fig. 6). It will also be observed that the checks lie at an angle to the longitudinal axes of the tracheids, forming a spiral around the cells (pl. 11, fig. 5).

In general, the line of demarcation between the two types of cells was very sharp where the compression wood was first laid down, but it blended gradually into the normal cells as it disappeared. All gradations of the two types of cells were found in the outer limits of the layer. The compression wood cells often had greater radial diameters and larger lumina than the adjacent summer-wood cells on either side (pl. 11, fig. 4), but this was not always the case since compression wood cells were often similar in shape and size to the ordinary cells, differing only in the structure of the secondary thickening (pl. 12, fig. 3). More highly developed compression wood cells, however, were rounded, leaving interstitial spaces (pl. 11, fig. 6).

When cross-sections of these cells were observed under high magnification (pl. 12, fig. 1), it was found that instead of concentric rings (lamellae) there were radial bands running from the region of the middle lamella to the lumen of the cell as described by Hartig ('96). These bands showed even more clearly under the Spierer lens (pl. 12, figs. 2 and 3) and their outlines and direction may also be observed with the cardeoid dark-field condenser (pl. 12, figs. 4 and 5). Although usually radial they were occasionally at an angle to the radius and were often irregular and crooked as can be seen in the illustrations. All gradations from the concentric laminae of the middle lamella to the typically radial bands shown in pl. 12, fig. 1, were found. The region of the middle lamella had the same

concentric arrangement as the cells illustrated in pl. 11, fig. 1, thus making a distinct demarcation between the middle lamella and the secondary thickening (pl. 11, fig. 6). These radial bands in the secondary wall of the compression wood cells appeared then to be the lamellae so distorted and displaced that they were no longer concentric cylinders but discontinuous radial bands. At frequent intervals they separated, forming checks or cracks in the cell wall, which usually extended only through the secondary thickening. It can readily be seen that on seasoning stresses would be set up not only between the compression wood and ordinary cells but also between the middle lamellae and secondary thickenings of the same cell.

When longitudinal sections of similar compression wood cells were observed under the Spierer lens, the alternating light and dark lines were found to be running in a spiral around the fiber, similar to the checks, except in the region of the middle lamella (pl. 12, fig. 6). The same phenomenon was observed when these cells were examined under the dark field (pl. 12, fig. 7). Here, as in pl. 11, fig. 5, the spiral lines between the checks were visible, showing the identical structure which is illustrated more clearly by the Spierer lens (pl. 12, fig. 6). These sections were cut tangentially through the secondary thickening of the cell wall, and the lines are the edges of the radial laminae. Here again the middle lamella is differentiated from the secondary thickening. In the former the laminae lie parallel to the axis of the tracheids, whereas in the latter they lie in a spiral. Then, in addition to being radial, the laminae of the compression wood cells are spiral plates which may be likened to the threads in a fine screw.

In the literature the terms "compression wood" and "*Rothholz*" are confined to the reddish cells found on the under side of leaning trees and the lower side of branches, characterized by their high density and by interstitial spaces. Koehler ('30) stated, however, that "In loblolly pine and redwood relatively high longitudinal shrinkage has been found in second-growth trees with very wide annual rings containing summer wood which is not so dark and hard as in normal wood.

Such wood resembles 'compression wood' in some respects but differs from it in extending entirely around the tree trunk and in some microscopic features.'' The microscopic features were not described by Koehler.

In the present study this type of wood was abundant in the more rapid-growth *Pinus Taeda* and was found in limited quantities in the other two species. It occurred on opposite sides of the same cross-section, in isolated areas, and as individual cells. On examination it was found to have the radial laminae spirally arranged in the cell wall which were characteristic of the typical compression wood or *Rothholz* cells. Therefore, it is evident that the old terms must be modified or replaced so that they will include all cells of a similar structure regardless of color or position.

Accordingly, the writer is suggesting a new term: *torquimural* (from the Latin *torqueo,* to twist or distort, and *murus,* wall). This term is based upon the structure of the cells and not upon their occurrence, which makes it applicable to any cell having this type of structure. Torquimural cells (as found in the southern yellow pines) may be defined as *tracheids with or without interstitial spaces, moderately to very dense, the secondary wall of the individual cell differentiated from the primary wall by its radial bands or laminae which lie in a spiral around the cell.* This term is not necessarily intended to replace the older terms but to describe a definite type of cell which is characteristic of compression wood or *Rothholz* but not limited to it.

In order to differentiate the torquimural tracheids from those having concentric laminae it becomes necessary to define the latter with a corresponding term based upon the structure of the cell wall. The term *concentrimural*[6] (concentric, having a common center, and the Latin *murus,* wall) is suggested. Concentrimural tracheids may be defined as *cells usually without interstitial spaces, thin- or thick-walled, the secondary thickening and the middle lamella of which are made up of layers or laminae in the form of concentric cylinders.* These

[6] The writer wishes to acknowledge the assistance of Mr. J. A. Moore, a graduate student in plant anatomy at Washington University, in selecting these terms.

terms will be used in the ensuing discussions where the two types of cells are compared or contrasted.

The Nature of the Failure and the Distribution of Strength in the Tree

COMPRESSION FAILURES

The nature of the failure of a small specimen of clear southern yellow pine wood subjected to pressure parallel to the grain depended upon (1) the density of the material, (2) the type of cells present (torquimural or concentrimural tracheids), and (3) the moisture content. In general, the plane of the major failure was in the direction of a tangent to the annual rings (pl. 13, fig. 1), although occasionally it occurred across the rings. Thil ('00) attributed the failure along the rings rather than across them to the supposition that the wood rays were arranged in spiral rows around the tree and that the plane of the spiral was weak. Fulton ('12) and Forsaith ('21) observed that the initial cause of all failure lay in the medullary rays. The displacement of fibers around the medullary rays, the reduction in diameter at this point, and the poor cohesion between the fibers and the rays caused a point of weakness. Robinson ('21) did not attribute the initial failure in spruce to the effects of the rays, but in ash and to some extent in pitch pine the rays were effective in causing failure. Jaccard ('13) denied the existence of spiral rows of rays as reported by Thil and expressed the belief that the rays were more resistant to pressure than the fibers, that the rupture came within the ray and not between it and the fibers. He did, however, recognize the curvature of the fibers around the rays, but stated that the failure was without constant relation to the rays. Bienfait ('26) suggested that the rays added strength in the radial direction and that the failure in the tangential direction was possibly due to this stiffening effect.

Tiemann ('06), from his studies of seasoned red spruce and chestnut, suggested as a general rule that "all species of wood, which when dry show the first indication of failure under compression by a crinkling of the cell walls without bending of the fibers, would be rigid, brittle, difficult to bend without break-

ing, and would increase rapidly in strength with dryness, whereas species which show a tendency for the fibers to buckle without the crinkling of the cell walls would exhibit the opposite qualities."

It is evident that Tiemann's observations were limited because the two types of failure may occur within the same growth ring and perhaps in the same cell at different degrees of dryness. Spring wood of rapid-growth material (which had large lumina and very thin walls) and brash wood (with an unusually high percentage of lignin or whose internal structure was unusual, such as torquimural cells) often crinkled or folded up, whereas concentrimural cells with thick walls usually buckled, unless the moisture content was very low. The secondary thickening of the thin-walled spring wood cell often broke loose from the middle lamella and folded into the lumen of the cell (pl. 13, fig. 2).

In specimens of green southern yellow pine under compression parallel to the grain, numerous wrinkles or offsets indicating failure were observed throughout the specimen. If the compression was continued one or more definite zones of failure developed, offsetting the specimen in one direction or forming a wedge split (pl. 13, fig. 1*b*). After the failure localized, most of the deformations over the remainder of the surface disappeared. Within this zone of failure the cells buckled, separating from each other or remaining in groups. These groups usually consisted of the tiers of fibers. In separating, the cell walls ruptured at the interface of the secondary thickening and the middle lamella, leaving the latter attached to one of the cells. In adjacent tiers of cells, the middle lamella often pulled away from the cell walls of one tier and remained attached to the other (pl. 13, fig. 2). This condition would be expected if the lignin of the middle lamella was somewhat stiffer and more brash than the cellulose walls (Dadswell and Hawley, '29).

The zone of major failure (plane of buckled cells) varied in size with the material tested. In tough green wood it often extended more than an inch along the specimen, and when the loading was continued after failure a large portion of the spec-

imen was macerated or shredded. In general the entire mass of cells buckled in one direction (pl. 13, fig. 1*c*), but when longitudinal sections of this region of failure were examined under the microscope it was found that individual cells at various points buckled away from the larger wood rays, the cells on either side buckling in opposite directions (pl. 13, figs. 3 and 4). The natural curvature in the tracheids at the point where they pass the larger wood rays, particularly the fusiform rays, and the reduction in diameter were sufficient to cause a point of weakness. The rays running in the radial direction cause a line of cells on either side of each ray to be curved in one direction. There were from 25 to 100 fusiform rays per sq. cm. and from 2000 to 3000 linear rays per sq. cm. in the pines studied here; therefore it is the opinion of the writer that the curvature of the tracheids was the controlling factor in the direction of the failure. This was further substantiated by the fact that in a number of the specimens failing *across the rings* the tracheids were found to be crooked and the greatest curvature was in the radial direction. So far as the southern pines reported here are concerned, the rays were effective in the position and direction of the initial failure. However, there was no definite arrangement of the rays observed as assumed by Thil.

When the individual buckled cells were examined by means of the Spierer lens, it was found that the inner wall (in relation to the curve) had folded or wrinkled, whereas the outer wall was drawn tight (pl. 13, fig. 5). In the torquimural cells the walls gave way entirely (pl. 13, fig. 6) or folded up by a shredding of the spiral laminae.

The "slip planes" in the individual cells observed by Robinson ('21) and Bienfait ('26) were, in the opinion of the writer, due either to the folding of the cell walls in the region of the pits as described by Tiemann ('06) or to an artifact brought about by the rupture of the laminae which exposed the amorphous lignin and pectin and caused differential staining. Similar slip planes may also be observed in torquimural cells where the laminae separate, forming checks.

Tracheids were often observed to buckle into the resin ducts

and thus become more completely separated from each other. In conclusion, it may be considered that an excess of large rays and resin ducts would reduce the strength per unit weight, but this effect was often overshadowed by crooked and otherwise inferior fibers.

STRENGTH AND STRUCTURE

The strength tests reported in this paper were made in the civil engineering laboratory at Washington University, with the assistance of Mr. Charles O. Quade and Mr. Chester Abbey[7] and under the direction of Prof. A. W. Brust, assistant professor of civil engineering.

These tests consisted of static compression parallel to the grain made on specimens 1½ x 1½ x 6 inches (specimens having a nominal length 4 times the least dimension). Since the object was primarily to determine the causes of variations in strength of material having a given density and percentage of summer wood and the distribution of strength in the tree, it was more desirable to use smaller specimens than those of a size specified by the American Society for Testing Materials (pieces 2 inches square). By using the smaller specimens it was possible to divide the cross-sections of the trees more nearly into their natural zones: inner heartwood containing short tracheids and numerous small rays, intermediate and outer heartwood containing long tracheids and larger rays, and outer sapwood. This emphasized the great differences in strength caused by differences in density and fiber characteristics in these various zones.

The compression tests were made on a 3-screw Riehle 150,000-lb. universal type testing machine at a cross head speed of 0.018 in. per minute.

Tables v (green material) and vi (seasoned material) show by bolts the number of tests, the average specific gravity, percentage of summer wood, rings per inch, and compression strength of the 7 trees used in this study. The average percentage of moisture was omitted in table v because it was above

[7] American Creosoting Co. Fellows in the department of civil engineering, Washington University.

the fiber-saturation point and had no significance, but it was included in table VI, which represents the seasoned material. The averages given represent all the tests made on the bolts used in the microscopic studies except a few individual tests from other bolts which showed unusual strength or weakness per unit weight. The corrected specific gravity[8] and measured

TABLE V

RESULTS OF COMPRESSION TESTS AVERAGED BY BOLTS AND BY TREES—GREEN. THE AVERAGE STRENGTH IS BASED ON THE AREA REPRESENTED BY THE TESTS MADE AT VARIOUS DISTANCES FROM THE PITH

Tree and bolt No.	Species	Number of tests	Original specific gravity	Corrected specific gravity	Estimated percentage of summer wood	Measured percentage of summer wood	Rings per inch	Maximum stress #/sq.in.
5-b	*Pinus Taeda*	53	0.549	0.521 (22)*	49	57	7.8	3570
5-i	(loblolly pine)	49	0.459	0.452 (30)*	29	38	9.4	3328
5-q		41	0.426	0.409 (25)*	24	28	8.8	2594
	Ave.		0.478	0.460	34	41	8.6	3164
7-a	*Pinus Taeda*	15	0.491	0.485	48	50	5.7	3540
7-g	(loblolly pine)	12	0.452	0.446	35	44	5.1	3450
7-m		11	0.436	0.428	25	36	5.8	3360
	Ave.		0.459	0.453	36	43	5.5	3360
3-a	*Pinus echinata*	15	0.602	0.588 (14)*	36	53	10	4625
3-c	(shortleaf pine)	11	0.544	0.535 (10)*	33	46	12	4477
3-e		11	0.519	0.510 (10)*	35	43	11	4110
3-h		11	0.475	0.465	29	38	13	3840
	Ave.		0.535	0.524	33	45	11.5	4263
4-a	*Pinus palustris*	22	0.565	0.509 (21)*	38	49	20	3752
4-c	(longleaf pine)	20	0.572	0.541	36	46	21	4003
4-f		15	0.555	0.509	40		20	3669
4-h		14	0.521	0.473 (13)*	29	38	21	3293
	Ave.		0.553	0.508	35		20.5	3679
6-a	*Pinus palustris*	12	0.569	0.542	44	44	24	4063
6-e	(longleaf pine)	9	0.571	0.489	39	34	22	3977
6-i		9	0.477	0.453	32	29	21	3172
	Ave.		0.539	0.494	38	36	22	3737

* The number of specimens represented in the corrected values.

[8] The specific gravity after correcting for resin content. These corrected values were used in all interpretations in this paper.

TABLE VI

RESULTS OF COMPRESSION TESTS AVERAGED BY BOLTS AND BY TREES—SEASONED. THE AVERAGE STRENGTH IS BASED UPON THE AREA REPRESENTED BY THE TESTS MADE AT VARIOUS DISTANCES FROM THE PITH

Tree and bolt No.	Species	Number of tests	Percentage of moisture	Original specific gravity	Corrected specific gravity	Estimated percentage of summer wood	Measured percentage of summer wood	Rings per inch	Maximum stress #/sq. in.
1-a	*Pinus Taeda*	25	6.6	0.589	0.569 (17)*	57	55	7.7	10677
1-d	(loblolly pine)	22	7.0	0.500	0.499 (17)*	34	38	7.5	9191
1-h		28	7.9	0.473	0.435 (12)*	29	30	6.9	8658
	Ave.		7.1	0.521	0.501	40	41	7.4	9508
2-a	*Pinus Taeda*	28	8.0	0.596	0.576 (20)*	52	53	5.4	11692
2-d	(loblolly pine)	24	6.2	0.508	0.488 (14)*	43	41	4.5	9512
2-h		24	7.7	0.495	0.479 (22)*	30	35	4.0	8775
	Ave.		7.3	0.533	0.514	41	43	4.6	9993

* The number of specimens represented in the corrected values.

summer wood[9] are given where a sufficient number of tests was made to justify a comparison. Where there were fewer tests the number is given in parentheses followed by an asterisk. The estimated summer wood was on the whole lower than the measured values.

These averages show particularly the variations in density and strength at the different heights in the tree. They also show to a certain degree the differences in strength shown by the various species. In the green condition *Pinus echinata* was consistently stronger than *Pinus palustris* which in turn was stronger than *Pinus Taeda* except for one bolt in tree 4 (fig. 3). The strength decreased from the base towards the top of the tree. The strongest material was in the lower bolts except in tree 4 where the strength reached a maximum in bolt *c* about 12 feet from the ground. In the lower part of the tree the strength fluctuated from bolt to bolt, but the maximum was invariably in the lower 12 to 15 feet in all three species. However, the maximum strength per unit weight was often

[9] The summer wood as measured under the microscope. Only the measured summer wood is given in the other tables and the text.

between the 20- to 40-foot level in the trees. The seasoned material of trees 1 and 2 showed the same relationship (fig. 3).

Tables VII (green material) and VIII (seasoned material) show results of individual tests and microscopic studies taken within each bolt. They show particularly the variations in properties which occur from the pith to the bark and to a cer-

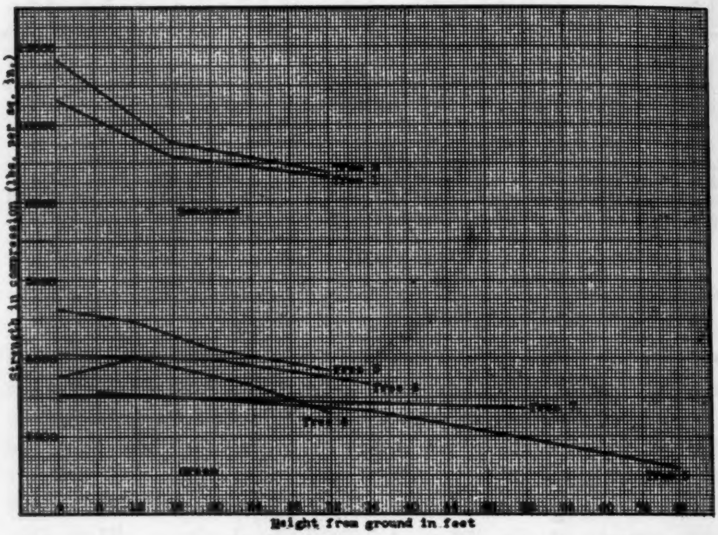

Fig. 3. The variation of strength with height in tree: below, the five trees tested green (trees 5 and 7, *Pinus Taeda*, loblolly pine, tree 3, *Pinus echinata*, shortleaf pine, and trees 4 and 6, *Pinus palustris*, longleaf pine); above, trees 1 and 2 (*Pinus Taeda*, loblolly pine) tested in the seasoned condition. The average strength of each bolt was obtained by evaluating the tests by the area represented in the cross-sections (tables V and VI).

tain extent on the opposite sides of a cross-section. In order to obtain a sample of the average material for microscopic study at least one series of specimens from the pith to the bark on the north side of each bolt was used. The north side was arbitrarily chosen since no one side was consistently weak or strong. In addition, the percentage of resin was determined for a large number of specimens on the other three sides of each bolt, and the specific gravity (corrected for resin content)

plotted against the strength. The specimens showing unusual strength or weakness were then studied microscopically. By this method material having average or very high strength and very low strength for its specific gravity was included. More material would necessarily have included a greater num-

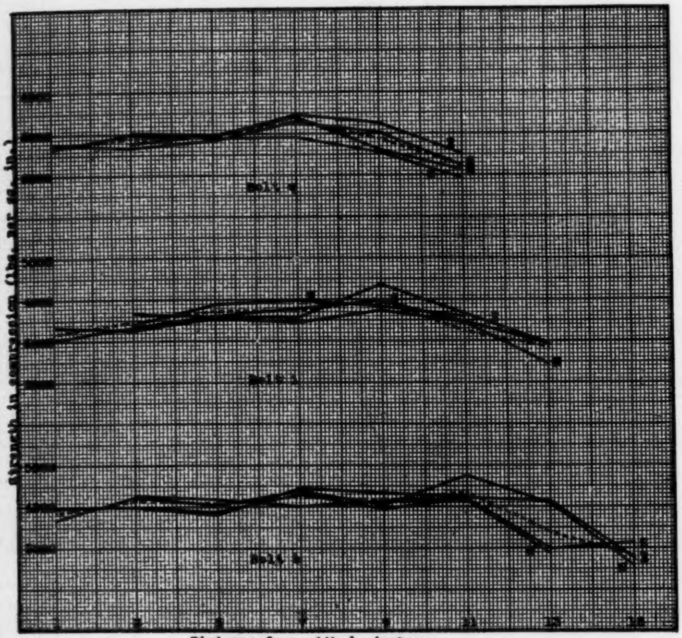

Fig. 4. Tree 5 (*Pinus Taeda*, loblolly pine), green. The solid lines represent the compression strength plotted against the distance from the pith for a single series of tests; the broken lines, the averages of all tests. Note how closely the average of all tests represents those plotted.

ber of variations but the maximum range for the material represented was covered.

In the cross-section of tree 5 *(Pinus Taeda)* the strength increased from the region of the first annual rings towards the bark. It reached a maximum at about 11, 9, and 7 inches from the pith in bolts *b*, *i*, and *q*, respectively, after which it decreased to a minimum in the outer sapwood (fig. 4). The tra-

TABLE VII

RESULTS OF MICROSCOPIC STUDIES AND RESIN DETERMINATIONS, TOGETHER WITH THE COMPRESSION STRENGTH, OF THE INDIVIDUAL SPECIMENS OF FIVE TREES—GREEN

Specimen	Maximum stress #/sq. in.	Specific gravity*	Percentage of resin	Percentage of moisture	Percentage of summer wood†	Rings/in.	Tracheid length in mm.	Percentage of area taken up by rays	Percentage of area taken up by resin ducts
Tree 5—*Pinus Taeda* (loblolly pine)									
5bN1	3680	0.494	2.7	34	44	10	3.0	6.90	1.53
5bN3	4240	0.547	2.4	32	53	6	3.5		1.13
5bN5	4020	0.577	2.5	36	62	5	3.7		1.13
5bN7	4380	0.595	1.9	33	63	5	4.0	8.51	1.74
5bN9	4040	0.574	2.0	35	60	4	4.3		1.83
5bN11	4750	0.588	2.1		65	8	4.5		1.37
5bN12–1	4640	0.544	2.3	35	60	7			1.37
5bN13	4090	0.546	2.4	39	60	9	4.6		1.23
5bN15	2570	0.428	2.6	104	53	23	4.6	9.72	1.27
5bN16–1	2520	0.450	2.5	125	50	14			1.59
5bS1	3970	0.480	1.8	36	54	9			1.71
5bS5	3880	0.572	1.9	32	63	5			1.22
5bS10–1	4450	0.559	1.7	34		8			
5bS13	2990	0.481	1.3	85		9			
5bS16–1	3230	0.407	2.5	111		19			
5bE3	3990	0.571	8.0	29	52	6			1.20
5bE5	3840	0.584	1.7	35	57	5		8.20	1.06
5bS3–6	3110	0.488	1.0	52		11			
5bE15	2750	0.460	2.0	106	48	14			1.49
5bN3–3	4030	0.588	1.9	34	56	5		9.75	0.88
5bW1–3	2870	0.464	2.6	99		15			
5bN3–6	3050	0.483	2.4	89	51	13			1.48

5iN1	3330	0.420	1.2	31	19	6	2.6	6.34	1.31
5iN3	3250	0.440	1.8	32	31	5	4.0		1.14
5iN4–1	3610	0.462	0.4	33	38	5			1.24
5iN5	3520	0.466	1.7	30	42	6	4.3		1.17
5iN6–1	3760	0.451	1.6	33	40	6			1.25
5iN7	3440	0.458	1.6	33	42	6	4.5	7.76	1.19
5iN8–1	3450	0.442	0.7	34	43	6			1.29
5iN9	3720	0.469	1.0	34	41	7	4.8		1.22
5iN10–1	3930	0.451	1.0	34	43	8		7.96	1.16
5iN11	3450	0.485	1.3	97	44	9	5.1		0.92
5iN13	2450	0.404	1.5	144	34	25	5.2	7.74	1.67
5iS1	3000	0.403	3.2	29		5			
5iS3	3280	0.443	1.3	34		5			
5iS5	3590	0.460	1.1	34	32	6			1.06
5iS7	3780	0.464	1.0	34	40	6			1.12
5iS8–1	3990	0.496	1.1	32	41	7			1.49
5iS9	3960	0.489	1.0	34		8			
5iS10–1	4130	0.492	2.0	34	39	9			1.63
5iS11	3470	0.446	1.4	42		11			
5iS13	2910	0.429	2.2	145		21			
5iE3	3660	0.445	1.7	33	35	6			1.14
5iS3–1	3590	0.455	1.9	33	33	6			1.29
5iS3–3	3940	0.494	1.6	33	42	7			1.26
5iE9	4390	0.486	1.5	31	45	7			1.26
5iW3	3380	0.422	1.0	33	33	6			1.13
5iW5	3870	0.456	1.0	33	40	6			1.17
5iN3–2	3940	0.467	1.7	33	42	6			1.22
5iW7	3950	0.477	1.6	35	42	7			1.32
5iN3–3	3850	0.448	4.0	32	42	7		7.89	1.31
5iN3–4	3840	0.445	1.8	35	38	10			1.52

* The specific gravity as reported here was corrected for resin content.
† The percentage of summer wood was determined by measuring the rings under the microscope.

TABLE VII (Continued)

Specimen	Maximum stress #/sq. in.	Specific gravity*	Percentage of resin	Percentage of moisture	Percentage of summer wood†	Rings/in.	Tracheid length in mm.	Percentage of area taken up by rays	Percentage of area taken up by resin ducts
5qN1	2665	0.378	5.4	30	17	4	2.5	6.18	1.19
5qN3	2980	0.403	2.3	32	30	5	3.8		1.18
5qN5	2900	0.399	4.7	33	29	5	4.3	10.60	1.12
5qN7	2920	0.422	2.4	30	33	7	4.6		1.42
5qN9	2560	0.419	2.0	45	32	11	4.7		1.25
5qN11	1925	0.362	3.8	162	28	22	5.1	8.06	1.65
5qS1	2760	0.396	4.3	33		4			
5qS3	2660	0.390	2.3	32		5			
5qS5	2810	0.405	2.0	32		6			
5qS7	3295	0.445	1.4	32	33	7			1.46
5qS8–1	3390	0.436	1.0	33	38	9			1.08
5qS9	3030	0.432	2.7	72		9			
5qS11	2210	0.382	4.2	153		15			
5qS12–1	2135	0.375	3.5	182	28	12			1.38
5qE3	2785	0.416	2.6	35		6			
5qE5	2940	0.420	2.9	34		5			
5qE7	3460	0.429	2.7	32	35	8			1.18
5qE9	3245	0.436	2.5	34		9			
5qE11	2500	0.415	3.5	136	30	13			1.56
5qS3–5	2605	0.443	2.0	147	39	16			1.34
5qW3	3015	0.410	4.5	30		6			
5qW5	2965	0.406	2.4	32		6			
5qW7	3410	0.470	2.9	33		9			
5qW9	2625	0.412	4.0	117		12			
5qW11	2115	0.336	3.1	178		22			

Tree 7—*Pinus Taeda* (loblolly pine)									
7aN1	2805	0.437	1.4	39	34	5	3.0	7.98	1.12
7aN3	3245	0.449	1.9	39	40	4	3.9	9.06	1.20
7aN5	3390	0.468	1.0	128	40	6	4.5	8.94	1.29
7aN7	3695	0.500	1.1	107	49	7	4.8	7.81	1.25
7aS1	3565	0.497	1.7	36	52	5			1.03
7aS3	3450	0.504	0.8	109	50	6			1.03
7aS5	3480	0.	1.0		53	4			1.33
7aS7	3865	0.527	0.8	84	65	8			1.88
7aE3	3110	0.448	2.9	44	46	4			1.34
7aE5	3025	0.439	1.2	127	51	6			1.29
7aE7	3090	0.459	1.5	119	58	4			1.87
7aE9	3530	0.478	0.7	114	47	8			1.49
7aW3	3705	0.535	0.8	103	55	6			1.15
7aW5	3955	0.536	0.7	102	47	7			1.27
7aW7	3975	0.513	0.9	106	58	11			1.53
7gN1	3245	0.430	1.1	34	33	4	3.2	7.09	1.03
7gN3	3065	0.410	1.3	49	36	5	4.1	7.08	1.07
7gN5	3115	0.406	0.7	140	40	7	4.9	7.13	1.12
7gS1	2765	0.431	1.5	33	35	4			0.94
7gS3	3025	0.434	0.7	114	44	5			0.90
7gS5	3510	0.470	1.8	109	53	6			1.18
7gS7	3705	0.491	0.8	105	58	7		6.34	1.31
7gE3	2960	0.422	4.0	33	48	3			0.90
7gE5	2810	0.420	1.0	120	41	5			1.10
7gE7	3580	0.476	0.8	123	45	5			1.00

* The specific gravity as reported here was corrected for resin content.
† The percentage of summer wood was determined by measuring the rings under the microscope.

TABLE VII (Continued)

Specimen	Maximum stress #/sq. in.	Specific gravity*	Percentage of resin	Percentage of moisture	Percentage of summer wood†	Rings/in.	Tracheid length in mm.	Percentage of area taken up by rays	Percentage of area taken up by resin ducts
Tree 7 (Continued)									
7gW3	3580	0.483	1.3	106	46	5			0.95
7gW5	3725	0.482	1.3	120	50	7			1.33
7mN1	2915	0.399	1.4	45	28	4	3.0	7.50	0.92
7mN3	3050	0.426	1.0	147	36	9	4.1	7.60	1.18
7mN5	3420	0.455	1.2	134	44	10	4.8	7.43	1.37
7mS1	2955	0.399	1.5	34	18	4			1.03
7mS3	2945	0.418	8.2	33	34	3			1.07
7mS5	2835	0.415	1.0	126	32	4			1.03
7mS7	2990	0.437	1.0	137	44	7		8.04	1.29
7mE3	2890	0.427	1.0	136	36	5			1.05
7mE5	3490	0.465	1.0	130	48	10			1.64
7mW3	3185	0.421	0.7	77	37	4			1.03
7mW5	3335	0.451	0.8	124	42	6			1.15
Tree 3—*Pinus echinata* (shortleaf pine)									
3aN2	5850	0.614	2.6	29	58	13	4.0	5.87	1.24
3aN4	5720	0.612	3.3	29	58	8	4.2	6.26	0.72
3aN6	4240	0.580	3.6	74	63	6	4.6	6.04	0.73
3aN8	4260	0.543	3.3	83	52	10	4.9	6.37	0.71
3aN10	4150	0.542	1.4	93	51	14	4.9	6.50	0.97

3aS2	4760	0.528	3.0	32	44	11	3.1		0.80
3aS4	4400	0.549	3.3	54	45	9	4.2		0.92
3aS6	4140	0.536	2.4	97	46	14	4.7		1.04
3aE3	5900	0.684	1.3	30	59	14	4.1		0.84
3aE5	5370	0.685	3.5	33	57	15			0.94
3aE7	4950	0.700	2.1	30	61	9	4.3		0.94
3aE9	4760	0.598	3.0	32	57	13			1.08
3aW3	3920	0.526	1.9	103	45	15	3.8		0.92
3aW5	4240	0.537	2.8	84	49	15	4.2		1.01
3cN4	4960	0.557	2.6	33	47	11	4.7	5.82	0.71
3cN6	4780	0.566	2.4	34	48	8	4.9	6.33	0.92
3cN8	4280	0.555	0.9	96	46	9	5.0	6.23	0.69
3cN10	4380	0.531	0.9	109	45	10	5.1	6.30	0.70
3cS2	4260	0.493	3.0	30	44	18			1.07
3cS4	4150	0.506	1.5	91	48	13	5.0		1.07
3cS6	4120	0.527	1.7	107	48	18			
3cE3	5090	0.570	1.6	113	50	12			
3cE5	4600	0.557	1.6	111	50	10	5.1		
3cW3	3930	0.487	1.5	34	46	12			
3eN2	4220	0.506	3.0	40	50	15	3.8	5.94	0.83
3eN4	4660	0.529	2.9	45	48	9	4.7	5.83	0.80
3eN6	4210	0.518	2.6	82	40	11	4.9	6.27	0.89
3eN8	3950	0.508	2.5	95	44	10	5.0	6.85	0.78

* The specific gravity as reported here was corrected for resin content.
† The percentage of summer wood was determined by measuring the rings under the microscope.

TABLE VII (Continued)

Specimen	Maximum stress #/sq. in.	Specific gravity*	Percentage of resin	Percentage of moisture	Percentage of summer wood†	Rings/in.	Tracheid length in mm.	Percentage of area taken up by rays	Percentage of area taken up by resin ducts
Tree 3 (Continued)									
3eS4	4080	0.509	1.0	107	45	10			0.71
3eS6	4000	0.527	1.1	94	44	15			0.73
3eE3	3980	0.533	1.2	93	40	15			
3eE5	4180	0.537	0.9	100	48	10			
3eW3	3230	0.458	2.3	33	40	9			0.70
3eS4–1	3520	0.474	2.1	60	35	10			
3hN2	3830	0.447	3.9	29	23	11	3.8	6.08	0.89
3hN4	4070	0.500	1.5	88	40	10	4.8	5.96	0.75
3hN6	3780	0.486	1.6	108	39	10	5.0	5.82	0.60
3hS2	3290	0.402	3.9	32	14	11			0.78
3hS4	3680	0.451	3.7	47	44	12			0.83
3hS6	3840	0.473	2.4	109	39	21			0.74
3hE3	4110	0.470	1.0	31	41	12			
3hE5	4210	0.508	1.6	97	47	17			
3hE7	3970	0.501	1.0	100	43	14			
3hW3	3230	0.434	1.6	43	40	10			0.59
3hW5	3110	0.448	1.5	127	35	9			

Tree 4—*Pinus palustris* (longleaf pine)									
4aN1	4020	0.530	2.9	35		40			
4aN2	5330	0.607	6.7	29	46	33	4.4	6.46	1.07
4aN3	5230	0.593	8.0	29		20			
4aN4	5780	0.654	5.1	32	60	15	4.8	7.00	1.10
4aN5	4260	0.524	7.8	54		15			
4aN6	4900	0.577	12.0	34	45	16	5.0	7.17	1.21
4aN7	3420	0.531	1.9	110		18			
4aN8	3520	0.500	3.2	116	45	20	5.1	7.95	1.34
4aS1	4310	0.554	12.3	26	35	27			1.00
4aS2	4100	0.502	11.2	32		29	4.2		
4aS3	3020	0.508	2.3	124		19			
4aS4	3000	0.486	2.3	128		20	4.7		
4aS5	2740	0.499	2.4	114	45	15			1.57
4aS6	2830	0.468	4.6	120		11	4.8		
4aE3	4840	0.623	9.3	26	49	17	4.5		1.13
4aE4	2960	0.457	4.1	126		24			
4aE5	3560	0.490	3.0	118		20	5.0		
4aW3	3830	0.508	6.9	86		22	4.8		
4aW4	4260	0.543	9.3	39		19			
4aW5	3460	0.509	1.9	113		18			
4aW6	3600	0.554	1.1	99		14			
4cN1	4570	0.538	4.9	28	39	36			1.08
4cN2	4670	0.652	4.5	22	40	33	4.0	7.59	1.22
4cN3	4420	0.557	6.8	33	52	24			1.27
4cN4	4970	0.581	7.1	29	53	20	4.9	7.20	1.27
4cN5	3690	0.508	3.1	107	57	21			1.17
4cN6	3440	0.531	3.4	108	35	27	5.2	7.63	1.26

* The specific gravity as reported here was corrected for resin content.
† The percentage of summer wood was determined by measuring the rings under the microscope.

TABLE VII (Continued)

Specimen	Maximum stress #/sq. in.	Specific gravity*	Percentage of resin	Percentage of moisture	Percentage of summer wood†	Rings/in.	Tracheid length in mm.	Percentage of area taken up by rays	Percentage of area taken up by resin ducts
Tree 4 (Continued)									
4cS1	4440	0.564	3.9	29		28			
4cS2	4220	0.541	9.5	27		23	4.6		
4cS3	3920	0.503	10.6	69		19			
4cS4	3840	0.567	4.2	85		18	4.8		
4cS5	3990	0.502	2.2	104	51	13			0.89
4cS6	4000	0.525	1.3	103		12	5.1		
4cE3	5230	0.620	2.5	29		16	4.7		
4cE4	4440	0.545	10.4	25		15			
4cE5	3810	0.518	6.6	102		28	5.2		
4cE6	3440	0.491	5.3	116		20			
4cW3	4040	0.525	6.1	79		23	4.8		
4cW4	3720	0.475	6.3	86		25			
4cW5	4060	0.545	4.9	96		16	5.0		
4cW6	4040	0.536	5.2	97		14			
4fN1	4140	0.563	22.4	23		22			
4fN2	2320	0.545	29.1	23	37	29			1.17
4fN3	3460	0.497	2.2	77		22			
4fN4	3970	0.518	4.9	54	38	21			0.92
4fN5	3860	0.539	1.2	100		13			
4fS1	4170	0.541	17.3	27	38	25			1.14
4fS2	4160	0.542	11.0	28		16			
4fS3	3080	0.446	2.4	111		26			
4fS4	3150	0.460	1.4	116		22			

4fE3	4110	0.524	1.9	57		23			
4fE4	3220	0.462	2.4	94		26			
4fE5	3330	0.483	1.5	116		23			
4fW3	3690	0.504	2.7	81		18			
4fW4	3560	0.483	3.3	66		19			
4fW6	3820	0.538	0.9	97		14			
4hN1	3150	0.528	23.5	23	41	22		5.80	1.15
4hN2	3930	(0.601)		27	39	20	4.1		0.85
4hN3	2890	0.500	7.7	36	36	29		6.08	1.06
4hN4	3630	0.554	7.4	69	37	22	5.2		0.96
4hS2	3350	0.470	10.9	32		18	4.3		
4hS3	2960	0.448	4.8	125		21			
4hS4	3040	0.436	1.2	137	38	24	5.1		1.15
4hE3	3450	0.471	5.7	79		17			
4hE4	3030	0.418	4.1	127		23	5.0		
4hW3	3270	0.457	4.6	118		22	5.0		
4hW4	3180	0.438	8.1	83		28			
4hW5	3350	0.478	5.3	115		20			
4hW6	3300	0.471	3.5	65		16			

* The specific gravity as reported here was corrected for resin content.
† The percentage of summer wood was determined by measuring the rings under the microscope.

TABLE VII (Continued)

Specimen	Maximum stress #/sq. in.	Specific gravity*	Percentage of resin	Percentage of moisture	Percentage of summer wood†	Rings/in.	Tracheid length in mm.	Percentage of area taken up by rays	Percentage of area taken up by resin ducts
Tree 6—*Pinus palustris* (longleaf pine)									
6aN1	6300	0.620	8.6	25	47	42	4.4	6.14	1.45
6aN3	4250	0.479	4.1	91	45	12	4.7	6.34	0.90
6aN5	3570	0.507	3.0	107	39	17	4.8	8.26	1.82
6aS1	5365	0.575	5.0	30	38	44			1.48
6aS3	5830	0.580	8.2	35	43	39			1.81
6aS5	4270	0.536	1.9	97	46	10			1.15
6aS7	3770	0.498	1.5	115	35	20	4.9	8.11	1.41
6aE3	4040	0.514	1.9	95	44	16			1.14
6aE5	4285	0.542	2.6	97	45	22			1.87
6aW3	5830	0.622	10.9	26	59	30			1.39
6aW5	4190	0.522	2.9	86	45	12			1.10
6aW7	3655	0.513	2.5	107	39	21			1.59
6eN1	3800	0.520	4.5	32	22	36	4.0	5.79	1.69
6eN3	4800	0.529	6.2	30	36	41	4.7	5.70	1.51
6eN5	3830	0.488	4.7	72	33	16	5.0	6.96	1.32
6eS1	4000	0.493	3.1	88	36	15			0.66
6eS3	3290	0.468	3.4	126	30	18			1.24
6eS5	3540	0.480	2.5	126	35	16			1.49
6eE3	3565	0.473	2.5	105	32	14			0.96
6eE5	4740	0.555	1.7	95	52	13			1.08

6eW3	3670	0.482	5.2	85	28	25			0.99
6iN1	4265	0.525	15.7	27	26	38	4.5	6.03	1.32
6iN3	2675	0.403	9.2	100	19	19	4.8	6.33	1.07
6iN5	2755	0.407	4.0	157	25	26	5.0	8.41	1.65
6iS1	4825	0.495	11.8		45	17			1.01
6iS3	3840	0.506	3.3	65	33	14			0.69
6iS5	2865	0.409	3.9	155	23	21			1.55
6iE3	3480	0.457	4.8	80	31	13			0.73
6iE5	3425	0.472	2.0	124	33	22			1.20
6iW3	2745	0.404	2.3	148	25	16			1.03

* The specific gravity as reported here was corrected for resin content.
† The percentage of summer wood was determined by measuring the rings under the microscope.

TABLE VIII

RESULTS OF MICROSCOPIC STUDIES AND RESIN DETERMINATIONS ON THE INDIVIDUAL SPECIMENS—SEASONED

Specimen	Maximum stress #/sq. in.	Specific gravity*	Percentage of resin	Percentage of moisture	Percentage of summer wood†	Rings/in.	Tracheid length in mm.	Percentage of area taken up by rays	Percentage of area taken up by resin ducts
Tree 1—*Pinus Taeda* (loblolly pine)									
1aN1	6430	0.526	5.4	6.5	35	3			
1aN2	6750	0.402	11.9	8.4	29	3	2.9	8.92	1.32
1aN3	8830	0.493	2.3	6.8	55	10			
1aN4	8770	0.526	2.4	6.8	59	10	4.0	8.82	1.61
1aN5	9870	0.566	2.0	7.1	65	6			
1aN6	9210	0.562	2.1	7.0	59	6	4.3	9.41	1.68
1aN7	9300	0.606	0.9	7.1	70	7		10.25	1.62
1aN8	8720	0.597	2.3	7.2	65	6	4.6	10.29	1.89
1aS2	8730	0.593	3.7	6.0	38	4		9.16	1.27
1aS4	12460	0.652	3.0	6.2	68	11			1.58
1aS5	14370	0.701	1.2	6.7	64	11			1.62
1aS6	13620	0.653	2.7	6.6	60	9			1.60
1aE3	10000	0.653	3.0	6.9		10			
1aE5	10840	0.554	2.4	6.8		7			
1aE7	9500	0.506	2.7	6.7		8			
1aW4	9470	0.540	3.0	6.4	55	10			1.35
1aW5	13120	0.638	2.2	6.5	60	7			1.27
1dN1	6440	0.404	2.0	6.7	11	3			
1dN2	6320	0.402	2.3	7.3	11	3	2.9	9.35	1.25
1dN4	7760	0.452	1.2	7.2	40	7	4.2	7.94	1.10
1dN6	8580	0.501	1.3	7.3	46	7	4.8	8.32	1.05

1dS2	6620	0.441	3.8	6.7	11	3			1.26
1dS3	10280	0.501	1.2	6.6	43	6			1.26
1dS4	10430	0.541	1.8	6.8	50	8			1.30
1dS5	11960	0.571	1.0	6.9	50	11			1.26
1dS6	13100	0.615	1.6	6.9	57	11			1.72
1dE3	8570	0.482	1.4	7.5		10			
1dE4	10320	0.503	1.1	7.3	43	10			1.08
1dE5	10260	0.500	1.4	7.3	48	7		8.54	1.40
1dE6	9940	0.484	1.0	7.3	47	8		8.08	1.40
1dE7	8390	0.470	1.7	8.5		9	5.0		
1dW3	10920	0.509	1.1	6.9	45	10		7.34	1.40
1dW5	11250	0.562	1.2	6.6		11			
1dW6	11380	0.562	1.1	6.4	53	9			1.50
1hN1	7240	0.430	2.4	8.0		5			
1hN2	5950	0.382	5.5	8.2	12	3	3.0	7.99	1.26
1hN4	7010	0.440	1.4	8.4	33	5	4.2	7.85	0.82
1hN6	8190	0.476	1.5	8.2	37	7	4.9	8.03	1.24
1hS1	6390	0.428	5.2	7.8	21	3			0.88
1hS2	6790	0.438	2.9	7.7	21	4			1.02
1hS4	9660	0.491	1.5	7.5	44	6			1.09
1hS6	8510	0.458	0.8	7.6	43	9			1.20
1hE3	7600	0.444	1.1	7.9		6			
1hE5	8610	0.448	2.2	7.9		9			
1hW3	6690	0.468	1.9	7.7		5			
1hW5	10100	0.510	1.3	7.9		12			

* The specific gravity as reported here was corrected for resin content.
† The percentage of summer wood was determined by measuring the rings under the microscope.

Table VIII (Continued)

Specimen	Maximum stress #/sq. in.	Specific gravity*	Percentage of resin	Percentage of moisture	Percentage of summer wood†	Rings/in.	Tracheid length in mm.	Percentage of area taken up by rays	Percentage of area taken up by resin ducts
Tree 2—*Pinus Taeda* (loblolly pine)									
2aN2	6500	0.455	4.1	10.3	31	3	2.8	8.75	1.86
2aN4	11330	0.610	1.6	9.9	58	6	3.7	8.66	2.08
2aN5	13940	0.641	0.5	6.8	60	7			1.65
2aN6	12130	0.591	1.1	9.9	62	7	4.4	9.98	1.32
2aN9	13340	0.619	0.6	10.1	63	7	4.9	9.42	1.47
2aS1	6760	0.412	13.0	9.7	17	3			2.38
2aS2	9000	0.555	10.0	8.7	35	4			2.25
2aS3	8160	0.527	4.7	7.0	33	3			1.73
2aS4	9870	0.566	7.8	11.1	50	3			2.03
2aS5	12060	0.578	1.0	7.0	57	5			1.52
2aS6	12400	0.620	1.4	10.4	60	5			1.75
2aS8	12820	0.617	1.3	9.5	60	6			1.60
2aE3	11800	0.595	1.6	6.0	55	5			1.54
2aE5	11330	0.621	1.7	7.2	60	5			1.44
2aE6	9940	0.573	1.6	7.1	63	5			1.22
2aE7	10560	0.623	1.9	7.2	65	5		9.84	1.88
2aE8	9650	0.578	1.2	7.6	65	6			1.90
2aW3	11600	0.552	1.7	7.2	50	5			1.54
2aW5	13000	0.628	2.2	7.0	65	6			1.65
2aW7	13000	0.623	1.9	7.0	62	10			1.83
2dN1	6100	0.434	21.2	8.3	17	2			2.05
2dN2	7170	0.427	3.7	6.6	30	2	3.0	8.02	1.51
2dN4	7800	0.466	0.9	6.1	48	3	3.7	7.81	1.58
2dN5	8010	0.524	1.8	6.2	45	5			1.61
2dN6	8650	0.516	1.1	6.5	50	4	4.5	8.65	1.40
2dN8	8430	0.500	0.7	6.4	50	5	5.0	9.59	1.47

2dS2	6700	0.446	15.4	6.7	15	3			3.15
2dS4	10830	0.519	0.5	6.2	52	5			1.92
2dS6	12150	0.552	0.9	6.0	52	6		9.15	2.02
2dE3	8370	0.486	0.5	5.8		4			
2dE5	8980	0.533	0.8	5.9		4			
2dE7	9260	0.526	0.5	5.9		7			
2dW3	9960	0.514	1.2	5.5		4			
2dW5	12270	0.502	1.4	7.0	54	6			1.61
2hN1	6240	0.376	20.8	8.7	20	3			2.50
2hN2	7000	0.452	6.8	7.7	20	2	3.1	8.36	1.34
2hN3	8140	0.422	3.9	8.0	26	2		7.88	1.85
2hN4	7910	0.433	1.8	7.7	30	3	4.0	7.88	1.85
2hN5	9260	0.472	2.3	7.7	33	3			1.95
2hN6	8360	0.454	1.0	7.5	37	3	4.5	7.85	1.71
2hN8	10720	0.511	0.9	6.6	49	8	5.1	9.75	1.70
2hS1	6820	0.456	5.5	7.4	20	2			1.66
2hS2	7520	0.455	9.0	8.1	21	3			1.82
2hS3	9750	0.495	1.0	7.8	43	4			1.49
2hS4	9550	0.506	1.0	7.3	42	5			1.46
2hS5	10440	0.524	2.0	7.3	47	5			1.41
2hS6	10400	0.522	0.9	7.3	48	6			1.59
2hE3	7220	0.444	1.2	7.7	30	2			1.54
2hE4	6520	0.455	2.6	8.1	34	3			1.60
2hE5	7450	0.510	1.1	7.7	32	3			1.71
2hE6	7780	0.524	2.3	7.7	56	3		9.61	2.20
2hE7	7680	0.504	1.4	7.7	37	6			1.49
2hE8	7250	0.501	2.2	7.8	40	7			1.64
2hW3	8270	0.477	1.4	7.8		3			
2hW4	7970	0.428	1.8	8.8	32	3			1.86
2hW6	9720	0.533	1.0	7.6		6			

* The specific gravity as reported here was corrected for resin content.
† The percentage of summer wood was determined by measuring the rings under the microscope.

cheid length and the density increased with the strength to a maximum, after which the density decreased with the strength, but the tracheid length only fluctuated within small limits. The resin ducts and to a certain extent the area taken up by the rays increased in this weak material of the outer sapwood. There were certain fluctuations in strength on the opposite sides of the cross-section, as well as in side-matched specimens from the same side due to the presence of torquimural and crooked tracheids and an accumulative effect of minor defects and injuries caused by strain in the living tree, but any given series of specimens from the pith to the periphery, when plotted, resembled in general any other series from the same bolt or the average of all of them. In each of the three bolts (*b*, *i*, and *q*) there were 8 series of specimens tested from the inner portion of the tree to the periphery (see fig. 1 for the relative positions of the 8 series of specimens). Four series of tests were plotted in fig. 4, together with the average of all 8 series. The agreement was very close at all points except bolt *i*, 11 inches from the pith. In this tree the growth rate was medium to rapid for the first 7–11 inches from the center, depending upon the bolt, thus placing the region of greatest density and strength some distance from the pith. The outer portion was of very slow growth and low density and strength. Figure 5, for tree 7 *(Pinus Taeda)* shows an increase of strength from the pith to the periphery. This tree, being much younger than tree 5, did not show a decline of strength in the outer sapwood.

The average strength decreased from the inner specimens towards the periphery in bolt *a* of tree 3 *(Pinus echinata)* and remained practically constant in bolts *c* and *e*, with a slight increase in the same direction in bolt *h* (fig. 6). The growth rate was very slow in the first 2–4 inches in bolt *a*, after which it increased at a moderate rate, but at the higher levels the growth rate was more rapid in the inner heartwood and the region of greatest strength was therefore farther from the pith.

The wide fluctuations in strength at a given distance from the pith in this and the two following trees were due to the asymmetric growth and crookedness of the trees, which made it impossible to obtain side-matched specimens from the same

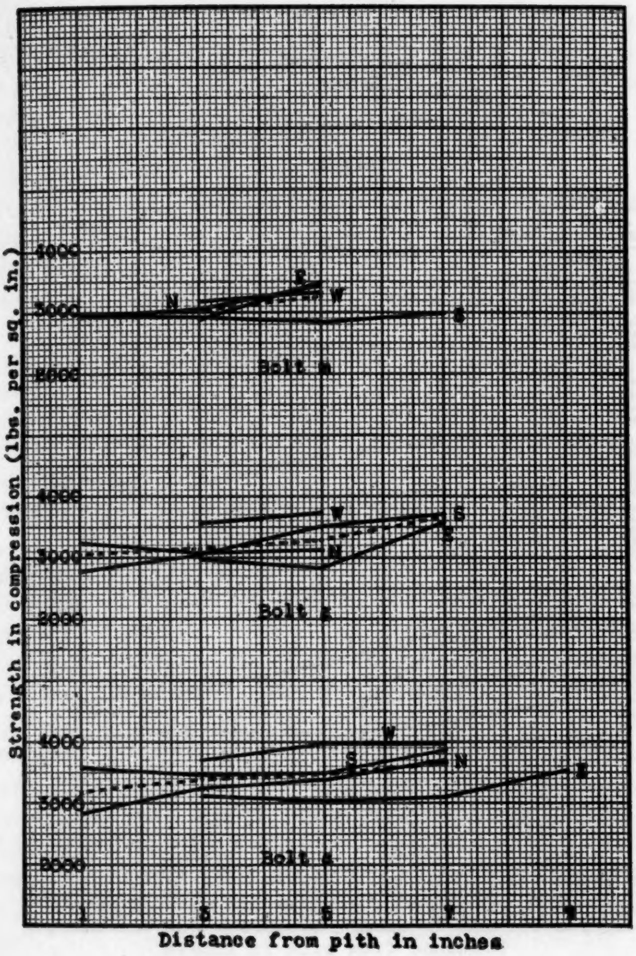

Fig. 5. Tree 7 (*Pinus Taeda*, loblolly pine), green. The solid lines represent the compression strength plotted against the distance from the pith for a single series of tests. The broken lines, the averages of these tests. Note that the average strength increased from the pith towards the periphery of the stem in all three bolts.

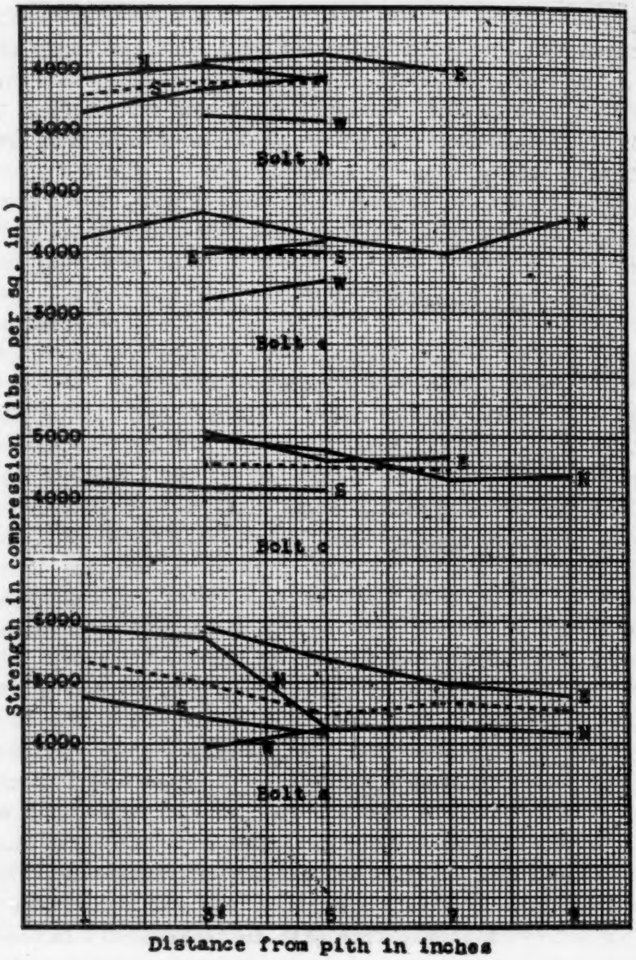

Fig. 6. Tree 3 (*Pinus echinata*, shortleaf pine), green. The solid lines represent the compression strength plotted against the distance from the pith; the broken lines, the averages of these tests. This tree was both crooked and asymmetrical, which accounts for the unequal number of tests on the different sides.

growth rings, as well as to the variations in the structure on the opposite sides. In the side having the slower growth the specimens would contain material laid down 20-40 years after that in the specimens on the opposite side, in the more rapid growth. Furthermore, areas of torquimural and crooked tracheids often occurred on one side, as opposed to straight concentrimural tracheids on the opposite side.

The *Pinus palustris* trees 4 and 6 were of very slow growth, especially for the first 2–4 inches along the radii (table I). In cutting the test sticks the pith and often the first rings containing the short tracheids, numerous resin ducts and wood rays were cut away. The result was that the maximum strength usually was within the first specimens around the pith. The average strength decreased then from this region towards the periphery of the stem (figs. 7 and 8), but occasional strong specimens occurred in the medium to outer portion of the trees. This was partly due to the impossibility of cutting directly through the pith in the crooked bolts, when the pith was thrown within a specimen which caused it to be weaker than its neighbor.

The seasoned material was of rapid-growth *Pinus Taeda* (trees 1 and 2), which increased in strength from the first formed growth rings towards the periphery of the stem. The outer sapwood in bolts *a* and *d* of tree 1 (fig. 9) had a lower strength than other specimens nearer the pith. The lower strength in the outer sapwood of tree 1 was due to the presence of a large percentage of torquimural tracheids and numerous large rays, particularly fusiform rays (tables III and IV), and not to a lower density (pl. 9, fig. 1, specimen 1aN7). The entire north and east sides of this tree, which were consistently low in strength, were composed of torquimural tracheids. There was a certain amount of this fiber throughout the stem.

Figure 11 shows the compression strength plotted against the specific gravity for the green material. The band[10] of points is thickest through the middle portion and thins out on

[10] Instead of trying to describe the strength-density relation as a straight-line relationship, the term "band of points" is used, which includes the deviation of the individual values from a straight line as shown in figs. 11 and 12.

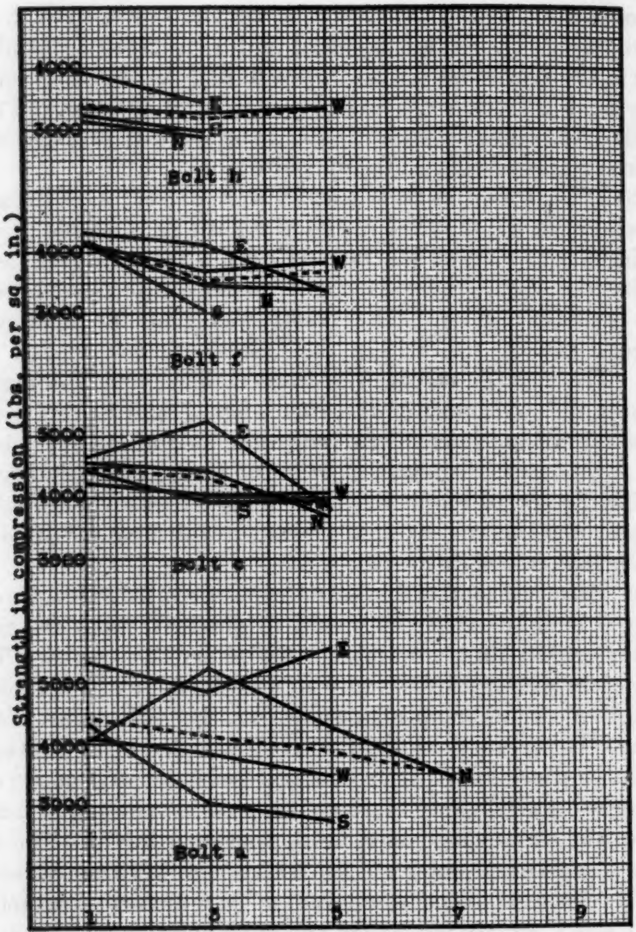

Fig. 7. Tree 4 (*Pinus palustris*, longleaf pine), green. The solid lines represent the compression strength plotted against the distance from the pith for one series of specimens on the various sides indicated; the broken lines, the averages of all tests made at the various distances along the radii.

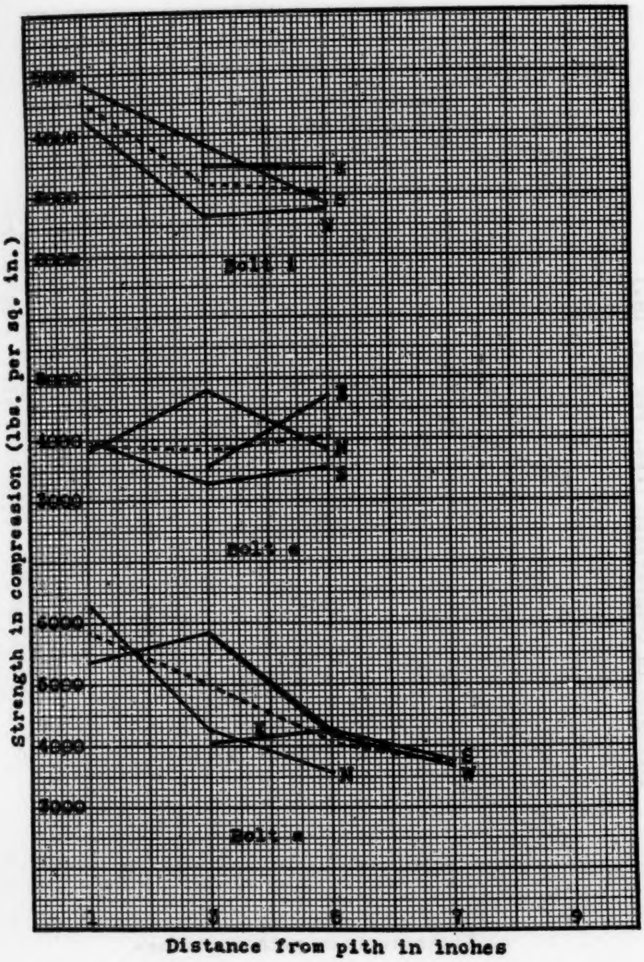

Fig. 8. Tree 6 (*Pinus palustris*, longleaf pine) green. The solid lines represent the compression strength plotted against the distance from the pith; the broken lines, the averages of all tests made in these bolts.

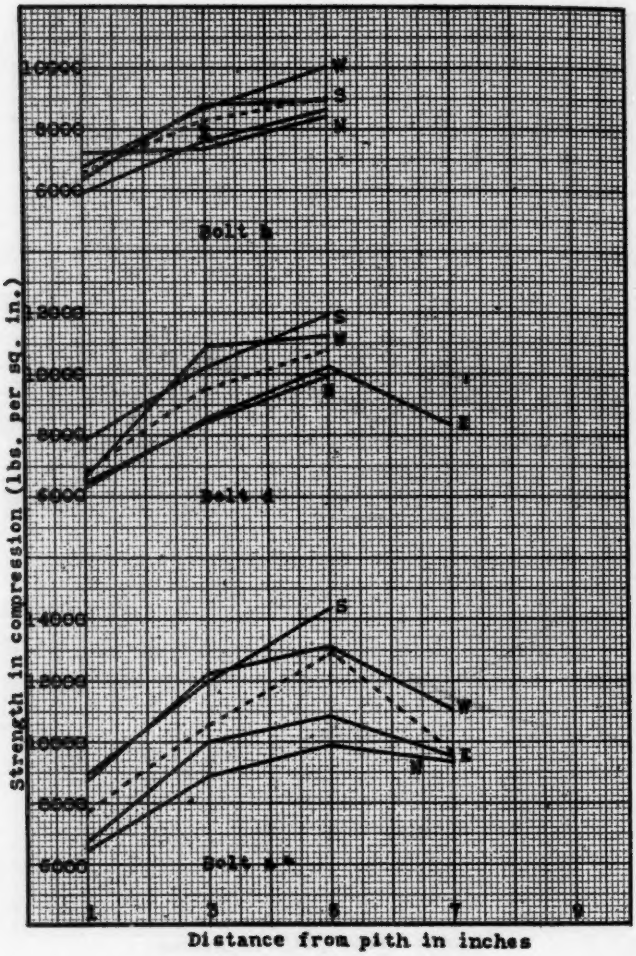

Fig. 9. Tree 1 (*Pinus Taeda*, loblolly pine), seasoned. The solid lines represent the compression strength plotted against the distance from the pith for a single series of tests; the broken lines, the averages of all tests made on each side.

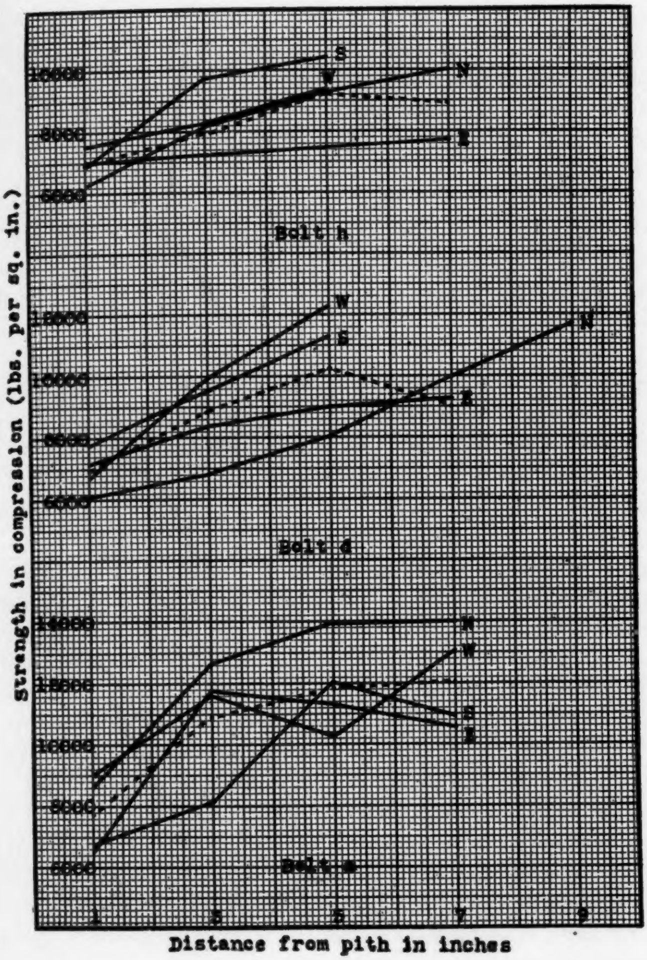

Fig. 10. Tree 2 (*Pinus Taeda*, loblolly pine), seasoned. The solid lines represent a single series of tests from the pith to the periphery of the stem on the four sides; the broken lines, the averages of all the tests made.

either side. The upper edge of the band composed of the strongest specimens per unit weight tends to form a straight line which is essentially parallel with the lower limit composed of the weakest specimens per unit weight and with the mean of all the points. In comparing the individual tests of *Pinus Taeda* and *Pinus palustris,* the former was the stronger for the lower range of specific gravity whereas for the higher range the latter was superior. *Pinus Taeda* seldom surpassed 4,000 pounds per square inch in strength, whereas the greater percentage of *Pinus palustris* was above 3,500 pounds. *Pinus echinata* covered practically the entire range of strength and density and was found in both the top and bottom edges of the band of points.

Table IX gives the strongest specimens for a given specific gravity, those found in the upper limits of the band in fig. 11, and the weakest specimens found on the opposite side of the same band. The stronger specimens had an average of 1,000 pounds greater strength than the weaker specimens, but at the same time 0.057 lower average density. The percentage of summer wood and the area taken up by resin ducts and wood rays were greatest in the weak specimens but the rings per inch and tracheid length were practically the same. In addition, a large number of the weaker specimens contained varying amounts of torquimural tracheids and all of them contained crooked or otherwise inferior tracheids (pl. 10, figs. 4 and 5), whereas the stronger specimens were made up of straight uniform concentrimural tracheids.

The seasoned material with a moisture content of from 7 to 8 per cent was about 2.6 times stronger than similar material in the green condition. Figure 12 shows the strength of the seasoned material plotted against the specific gravity. The same type of band was formed as in the green material. Table X shows the strongest and weakest specimens as found on the opposite sides of this band. The same conditions in the cells in the strong and weak material were found here as in the green material except that the torquimural cells were more pronounced, and the area taken up by resin ducts and wood rays was much higher in the weak specimens. The wood rays were

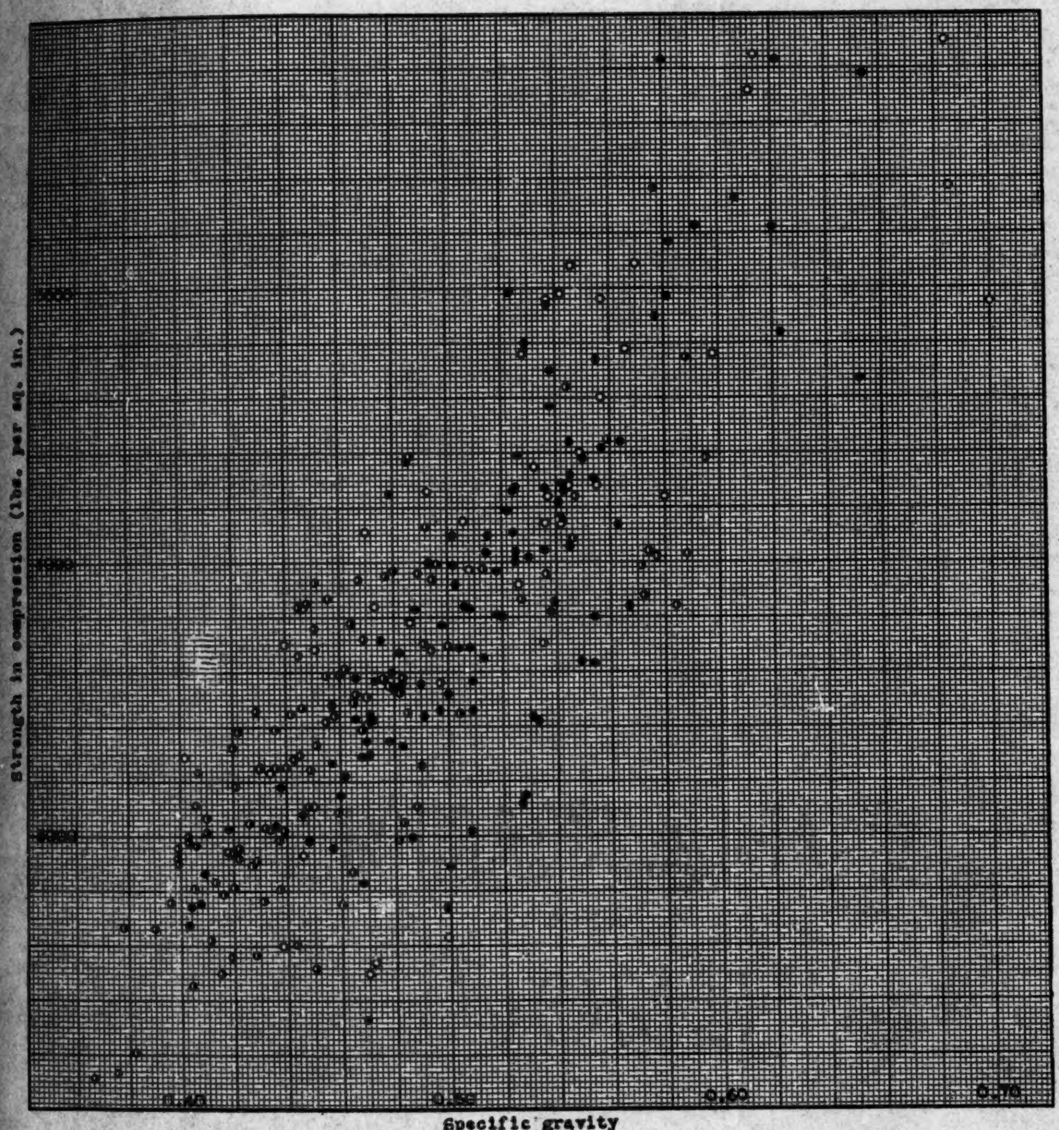

Fig. 11. The compression strength for the green material plotted against the specific gravity. *Pinus Taeda* (loblolly) is represented by a black-and-white circle; *Pinus echinata* (shortleaf), by a white circle; *Pinus palustris* (longleaf), by a black circle.

slightly larger and more numerous in areas containing a large percentage of torquimural tracheids, particularly the fusiform rays which were more effective in causing failures.

The poor differentiation of spring and summer wood in the early growth of a tree, and numerous rings in the later growth,

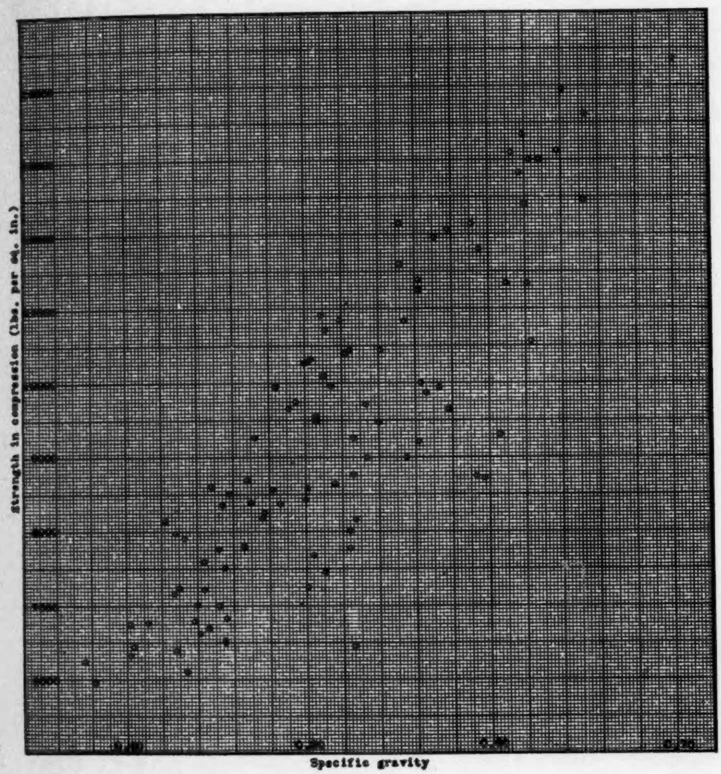

Fig. 12. The compression strength for the seasoned material, trees 1 and 2 (*Pinus Taeda*, loblolly pine), plotted against the specific gravity.

together with the differences in thickness of cell walls in both the spring and summer wood of the different species, caused a difference in the density of the material containing an equal percentage of summer wood. *Pinus palustris* had thicker-

TABLE IX

THE 15 STRONGEST AND THE 15 WEAKEST SPECIMENS PER UNIT WEIGHT TAKEN FROM FIG. 11—GREEN MATERIAL

Tree and specimen No.	Compression strength in lbs./sq. in.	Specific gravity	Percentage of resin	Percentage of moisture	Percentage of summer wood	Rings per in.	Tracheid length	Total area taken up by resin ducts and wood rays
Strongest specimens per unit weight								
6aS3	5830	0.580	8.2	35	43	39	4.7	7.88
6aN1	6300	0.620	8.6	25	47	42	4.4	7.59
6aN3	4250	0.479	4.1	91	45	12	4.7	7.24
3hS2	3290	0.402	3.9	32	14	11	3.8	7.74
3hN2	3830	0.447	3.9	29	23	11	3.8	7.07
3hE3	4110	0.470	1.0	31	41	12	4.8	7.18
3gN2	3700	0.440	2.2	29	38	10	3.9	7.07
3bS2	5080	0.546	2.0	32	55	19	4.0	7.36
3bS4	4950	0.537	2.3	54	51	19	4.2	7.36
4dN4	4370	0.485	4.3	46	43	20	5.0	7.71
4bN3	4940	0.537	4.6	39	40	23	4.7	7.99
5iN10–1	3930	0.451	1.0	34	43	8	5.1	9.59
5iN3–4	3840	0.445	1.8	35	38	10	5.0	9.41
5iN3–3	3850	0.448	4.0	32	42	7	4.4	9.20
5iE9	4390	0.486	1.5	31	45	7	4.8	9.06
Ave.	4440	0.491	3.56	39	41	16.6	4.5	8.62
Weakest specimens per unit weight								
3gS6	2630	0.499	2.9	109	46	20	5.2	7.37
3gS4	2500	0.470	2.9	47	33	18	4.8	8.11
3gS4–1	2540	0.472	3.4		32	12	4.9	7.89
3aE7	4950	0.700	2.1	30	61	9	4.3	8.62
4bN6	2330	0.469	3.5	115	41	26	5.0	8.98
4gW6	3120	0.527	3.1	109	40	21	5.1	8.69
4cN2	4670	0.652	4.5	22	40	33	4.0	8.81
4aS3	3020	0.508	2.3	124	45	19	4.7	8.65
4aN7	3420	0.531	1.9	110	45	18	5.0	9.34
4hN1	3150	0.528	23.5	23	41	22	4.0	8.95
4hN4	3630	0.554	7.4	69	57	22	5.2	8.51
5bE5	3840	0.584	1.7	35	57	5	3.7	9.26
5bN3–3	4030	0.588	1.9	34	56	5	4.6	10.63
5bS5	3880	0.572	1.9	32	63	5	4.0	9.57
5bN5	4020	0.577	2.5	36	62	5	3.7	9.53
Ave.	3448	0.548	4.37	59	48	16	4.5	8.86

walled cells with smaller lumina than either *Pinus echinata* or *Pinus Taeda*. It is indicated from fig. 13 that for a given specific gravity *Pinus echinata* must contain about 10 per cent and

TABLE X

THE 10 STRONGEST AND THE 10 WEAKEST SPECIMENS PER UNIT WEIGHT TAKEN FROM FIG. 12—SEASONED MATERIAL

Tree and specimen No.	Compression strength in lbs./sq. in.	Specific gravity	Percentage of resin	Percentage of moisture	Percentage of summer wood	Rings per in.	Tracheid length	Total area taken up by resin ducts and wood rays
Strongest specimens per unit weight								
2dS6	12150	0.552	0.9	6.9	52	6	4.9	11.17
1dW3	10920	0.509	1.1	6.9	45	10	4.3	8.74
2hN8	10720	0.511	0.9	6.6	49	7.5	5.1	10.59
1dE6	9940	0.484	1.0	7.3	47	7.5	4.8	9.49
2hN3	8140	0.422	3.9	8.0	26	2	4.0	9.73
1dE5	10260	0.500	1.4	7.3	48	7	4.8	9.94
2dS4	10830	0.519	0.5	6.2	52	5	3.7	10.97
1dE4	10320	0.503	1.1	7.3	43	10	4.3	9.03
1dS3	10280	0.501	1.2	6.6	43	6	4.0	9.16
1hE5	8610	0.448	2.2	7.9	40	9	4.9	9.21
Ave.	10217	0.495	1.4	7.0	44.5	7	4.48	9.90
Weakest specimens per unit weight								
1aN7	9300	0.606	0.9	7.1	70	6.5	4.6	11.87
1aN8	8720	0.597	2.3	7.2	65	8	4.6	12.18
1aS2	8730	0.593	3.7	6.0	38	4	2.3	10.43
1aN1	6430	0.526	5.4	6.5	38	3	2.9	10.30
2aE7	10560	0.623	1.9	7.2	65	5	4.5	11.72
2hE6	7780	0.524	2.3	7.6	56	3	4.8	11.81
2hE8	7250	0.501	2.2	7.8	40	7	5.1	11.34
2hE5	7450	0.510	1.1	7.7	32	3	4.3	11.35
2dN5	8010	0.524	1.8	6.2	45	5	4.5	10.21
2aE8	9650	0.578	1.2	7.6	65	6	4.9	11.40
Ave.	8388	0.558	2.28	7.0	51	5	4.2	11.26

Pinus Taeda 20 per cent more summer wood than *Pinus palustris*. This is further amplified by fig. 14, which shows the strength plotted against the percentage of summer wood. For the most part, each species occupied a different region of the graph, *Pinus Taeda* being weakest, *Pinus palustris* strongest, and *Pinus echinata* intermediate for a given percentage of summer wood. The overlapping of the species was greatly influenced by the variations in strength in a given species due to inferior fibers as described for the material represented in

figs. 11 and 12. When the strength of a single species was plotted against the percentage of summer wood, however, there was less spread of points and a band similar to the specific gravity-strength relations was obtained (fig. 15). Since the specific gravity (when corrected for resin content) is a more accurate measure of the mass represented, there was less spread in the points when the specific gravity was plotted against the strength (compare figs. 12 and 15). Essentially

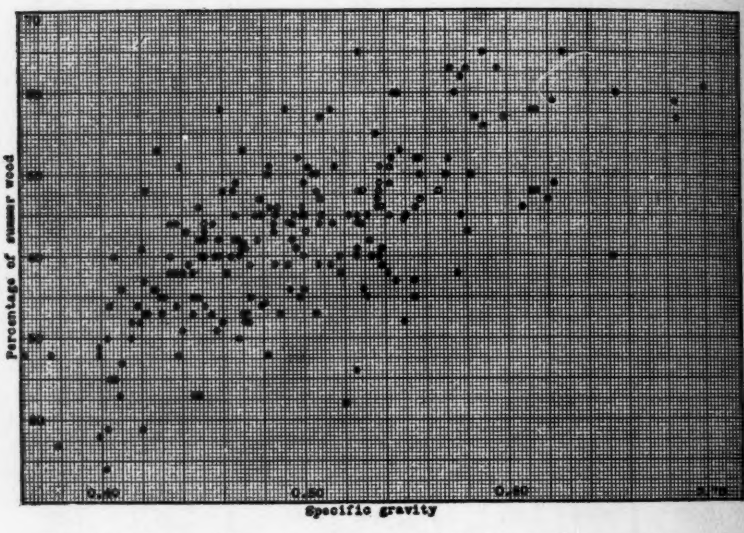

Fig. 13. The percentage of summer wood plotted against the specific gravity. *Pinus Taeda* (loblolly pine), black-and-white circle; *Pinus echinata* (shortleaf pine), white circle; *Pinus palustris* (longleaf pine), black circle.

the same specimens were found in the upper and lower limits of the bands in these two figures.

Discussion

The specific gravity of clear specimens of southern yellow pine wood is not an accurate measure of its strength, and the percentage of summer wood, without regard for the density of the different species, shows even greater variations under present specifications. Resins increase the specific gravity,

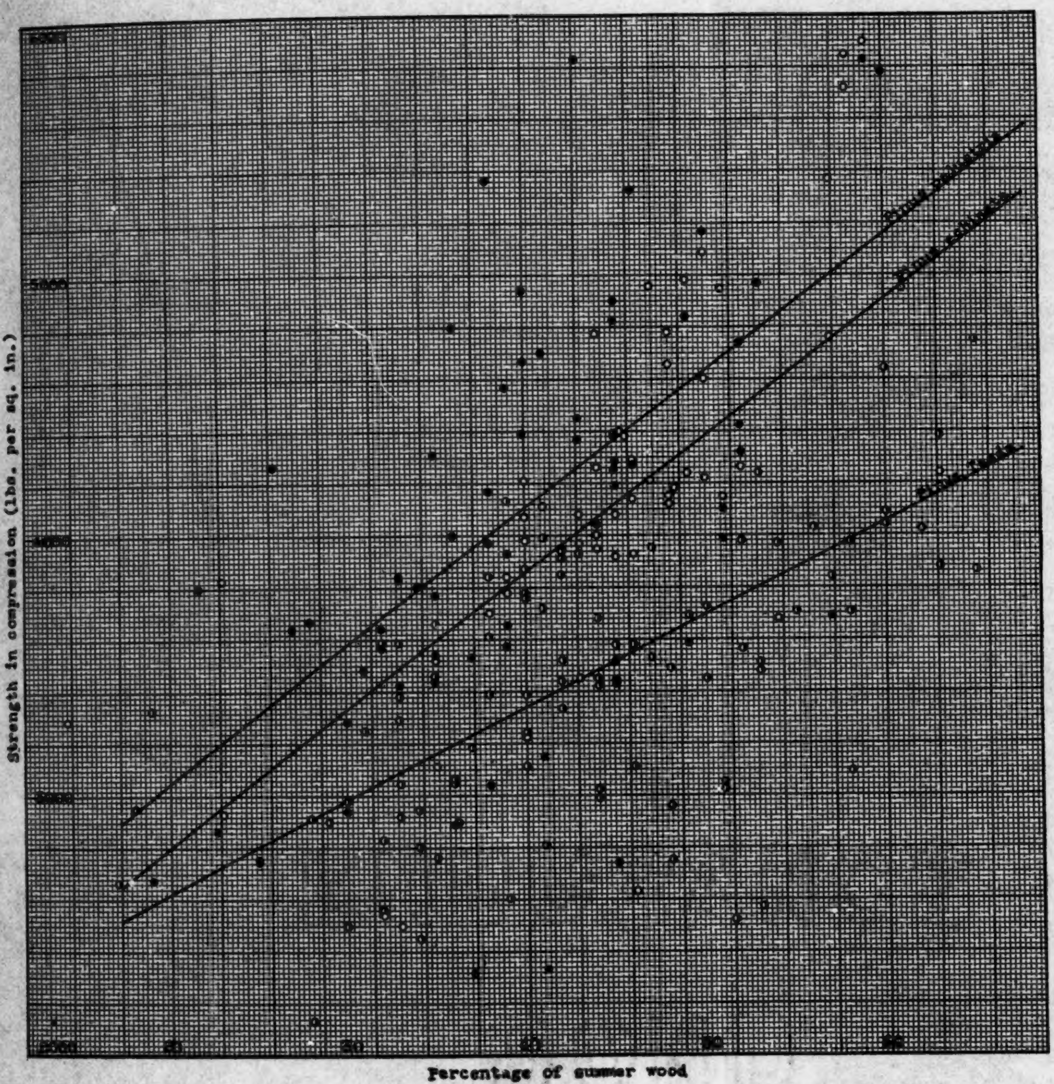

Fig. 14. Trees 5 and 7 (black-and-white circle, *Pinus Taeda*), tree 3 (white circle, *Pinus echinata*), trees 4 and 6 (black circle, *Pinus palustris*), green. The compression strength plotted against the percentage of summer wood. The lines represent the averages of the various species as indicated. The wide spread of points may be largely attributed to the differences in density of the summer wood in the three species as shown in fig. 13.

with little or no effect upon the strength. Although the wider variations were eliminated by correcting the specific gravity for benzol-soluble compounds, there were still variations of from 1,500 to 2,000 pounds per square inch for practically the

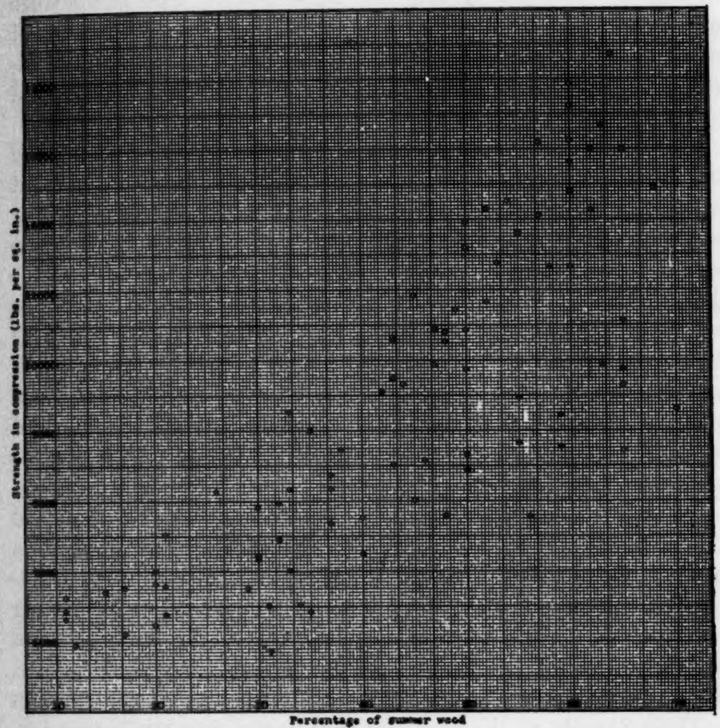

Fig. 15. Trees 1 and 2 (*Pinus Taeda*), seasoned. The compression strength plotted against the percentage of summer wood. Note the close agreement of this figure with that of fig. 12, which represents the compression strength plotted against the specific gravity for the same material.

entire range of specific gravity in the green material. In spite of these variations the strength of the best material varied directly with the first power of the density, and the average strength of the different species agreed very well with the averages reported by Newlin and Wilson ('17) for the same

species, with the exception of tree 3, *Pinus echinata*, which was unusually strong for its species. There was an increase of about 1,300 pounds per square inch for an increase of 0.1 in specific gravity in the green material and about 3,000 pounds in the dry material if only the most perfect specimens were considered. When the increase in strength for the changes of moisture was accounted for, both the green and seasoned material showed essentially the same relations of density and strength.

Since the variable due to moisture content was eliminated by using only green material (that with a moisture content above the fiber-saturation point) and specimens of a similar moisture, and since the absolute density of the wood substance was constant, the variations in strength were attributed to the differences in structure. The anatomical structure of the southern yellow pines is simple and has been described by Mohr and Roth ('97) and others. The woody cylinder or xylem is composed primarily of tracheids with occasional groups of wood parenchyma. An anastomosing system of resin canals and the wood rays make up the remainder of the structure.

In order to make any correlation between the strength and the structure, it was necessary to evaluate the strength on a unit-weight basis known as the specific gravity. The short-fibered material in the first few growth rings was weak, being less than half as strong in certain cases as the longer-fibered material farther out in the tree, but it was also much lighter. The very first rings, however, were not so strong in proportion to their density as the best material containing the longer tracheids. On the other hand, they were not so weak in proportion as the poorest material, which also contained long tracheids. The weakness of this short-fibered material was apparently due to the large number of resin canals and wood rays which interrupted the unity of the material. In very slow-growth trees this area may be neglected but in rapid-growth material there may be a difference of 50 to 100 per cent in strength along a radius of from 6 to 8 inches (table VIII, compare specimen 1dS2 with 1dS6 and 2aN2 with 2aN5).

As the tracheids increased in length the density also increased, but the latter reached a maximum between the ages of 40 and 100 years (also Mohr and Roth, '97), after which it declined more or less rapidly, whereas the tracheid length sometimes fluctuated although with little regular reduction in length. The maximum strength was found to be in this high density material in the intermediate zone, but the strength was often much lower than was indicated by the density. It was for the most part this high-density material that showed the greatest fluctuation in strength per unit weight.

Comparing the extremes in strength per unit weight, there was little difference in the length of the tracheids and rings per inch in the strongest and weakest specimens of the green material, but there were differences in the percentage of area taken up by resin ducts and wood rays and particularly in the contour of the cells and the structure of the cell walls. The percentage of area taken up by the wood rays and resin ducts was noticeably greater in the weak specimens of the seasoned material, but it is evident that these factors (fiber length, resin canals, and wood rays) were not entirely responsible for the wider variations in strength although it was indicated that they contributed to it. The fact that the tracheids buckled away from the rays (pl. 13, figs. 3 and 4) indicated that an excess of rays would be weakening. Furthermore, areas containing a high percentage of torquimural tracheids had a greater percentage of rays than the other material.

There was no direct correlation between the number of rings and the strongest or weakest specimens per unit weight, but the greatest strength for a given tree was usually found in the intermediate growth, while low density and low strength were associated with extremely rapid or slow growth. The weaker specimens per unit weight had a much higher percentage of summer wood than the stronger ones regardless of species, but in the material containing straight uniform tracheids with concentric laminae (concentrimural tracheids) the strength increased with the summer wood within a given species. In general, however, for a given specific gravity, *Pinus echinata* contained 10 per cent and *Pinus Taeda* 20 per cent more summer

wood than *Pinus palustris*, and similarly for a given strength there was a greater percentage of summer wood in *Pinus Taeda* and *Pinus echinata* than in *Pinus palustris*. From this it is concluded that for the strongest material per unit weight one must choose medium to low density *Pinus Taeda* and medium to high density *Pinus palustris*. This is borne out by fig. 11, which shows that for the lower density material *Pinus Taeda* is the strongest, whereas for the high density material, *Pinus palustris* and *Pinus echinata* are superior. *Pinus echinata* was not sufficiently well represented here to justify any definite statement as to how it would fit into this relationship.

Since the poorer material was not confined to the very dense or very light wood and since the greater variations in strength were not entirely due to the length of the tracheids nor the wood rays and resin canals, the cause must be sought in the general contour of the cells and the structure of the cell walls. Weakness was associated with torquimural, crooked, and otherwise deformed tracheids, although the walls of the latter were often made up of the usual concentric laminae. The crooked fibers tended to buckle more readily than the straight ones, which caused the material to be considerably weaker than was indicated by its density.

Sonntag ('04) found that the tensile strength of ordinary wood was from 25 to 50 per cent greater than that of compression wood (torquimural cells). He also stated that the compression side of the tree had a greater resistance to compression than the tension side due to the thicker walls, which gave a greater resistance to bending in that direction. He did not take into account the great differences in density, however, which more than offset the increase in strength or stiffness. Jaccard ('28) observed that compression wood contained a higher percentage of lignin than normal wood, as did Dadswell and Hawley ('29) for compression wood of Sitka spruce. Koehler ('33) reported that compression wood in the green condition was low in stiffness and on a unit-weight basis was inferior in all strength qualities. When seasoned the differences were much greater, and the compression wood was very brash in tension.

The torquimural tracheids, with an entirely different wall structure from the concentrimural cells, were brash and had from 1,500 to 2,000 pounds less strength per unit weight in the green condition and from 2,000 to 3,500 pounds less in the seasoned condition than the material made up of straight cells. The increase of strength with dryness was not so great in the specimens containing large quantities of torquimural cells, due primarily to the checking of the individual cell walls and to the internal stresses caused by unequal shrinkage. The middle lamella had the same concentric arrangement as the concentrimural cells and in most cases the latter were interspersed with the torquimural tracheids. This condition, together with the great differences in the pitch of the spiral of the laminae (and micells which are contained in the laminae) in adjacent torquimural cells, was responsible for these internal stresses. The low strength of the torquimural tracheids in the green condition was caused by two factors: (1) the structure of the cell walls, and (2) the poor union between the cells, due to the rupture of the middle lamellae forming interstitial spaces.

The great differences in strength per unit weight of green southern yellow pine may be largely attributed to the variations in the straightness of the tracheids, disruption of the middle lamellae forming interstitial spaces, the structure of the cell walls, and, to a certain extent, to the variations in resin ducts and wood rays (particularly fusiform rays). It must be borne in mind, however, that the effects of these factors were accumulative, that no single one of them alone, with the possible exception of the structure of the cell wall, was responsible for the wider variations in strength.

Due to the fact that one or more of the above factors was present and effective to a certain extent in any given sample of wood, it is evident that the density plotted against the strength is not a straight-line relation but a band relation, of which the more perfect specimens will be found in the upper portion and the spread depends upon the diversity of the material.

Although variations of from 50 to 100 per cent in strength of small clear specimens in southern yellow pine timber taken

from the same tree and similar but smaller variations in strength of specimens of the same density may be expected, this need not be an unsurmountable barrier to the grading and uses of this material for special designs. The knowledge of the variations in density and strength in the cross-section in relation to the age and growth rate of the tree and at the different heights in the tree will make it possible to cut timbers of a structural size without serious differences in strength. The knowledge of the differences in specific gravity for a given percentage of summer wood in the various species will make it possible to evaluate the strength more correctly on the basis of percentage of summer wood. The serious defects in the form of crooked fibers and torquimural tracheids are visible in dressed materials and may be eliminated in pieces of small dimensions. A more complete knowledge of the extent and distribution of these major defects in the virgin and second-growth timber of a given species and in different species of wood used for structural timbers will make it possible to modify the grading rules to take care of these variabilities.

The bending strength and elastic properties of this material, together with a more complete treatment of the compression strength as related to engineering practice, will be given in a future paper.

Summary

1. Compression tests parallel to the grain, together with a study of the physical properties and microscopic structural features, were made on small specimens of clear wood taken from seven southern yellow pine trees cut in Walthall County, Mississippi. Four of these trees were *Pinus Taeda,* one was *Pinus echinata,* and two were *Pinus palustris.*

2. The fiber-saturation point as measured by the electro-conductivity method was 22.5 ± 1 per cent.

3. The absolute density of the wood substance was 1.52 as compared with water at 4° C.

4. The percentage of benzol-soluble materials designated as resins varied from 0.4 to 21 per cent in *Pinus Taeda,* with an average of 2.3 per cent, from 0.9 to 6.4 per cent in *Pinus echi-*

nata, with an average of 2.5 per cent, and from 0.9 to 29.1 per cent in *Pinus palustris,* with an average of 5.6 per cent.

5. The rate of growth varied from 1¾ to 25 rings per inch in *Pinus Taeda,* from 5 to 21 rings in *Pinus echinata,* and from 9 to 55 rings per inch in *Pinus palustris.* The first-formed rings of the *Pinus Taeda* trees were very broad, the growth rate decreasing with an increase in the age of the tree, whereas in the *Pinus echinata* and *Pinus palustris* trees the first 2 to 4 inches from the pith were of very slow growth, the growth rate increasing with the age of the trees.

6. The percentage of summer wood, and thus the density, decreased from the region of the base of the tree towards the crown. In general, the density increased from the first growth ring to a maximum in the region of the 100th ring and declined from this point towards the periphery of the stem.

7. For a given specific gravity, *Pinus echinata* contained about 10 per cent, and *Pinus Taeda* 20 per cent, more summer wood than *Pinus palustris.* The differences in density were due to the variations in thickness of the cell walls.

8. The percentage of area taken up by resin ducts was greatest in *Pinus Taeda* and least in *Pinus echinata.*

9. The wood rays decreased in number and increased in size from the pith to the periphery of the stem in all three species. They occupied slightly more area at the base of the tree, decreased somewhat at the higher levels, and again increased to a second maximum at the top. They were largest in the rapid-growth *Pinus Taeda* and smallest in *Pinus echinata.*

10. The tracheid length increased rapidly through the first 10 growth rings and more slowly through the remaining rings. Slight fluctuations in length were observed in the outer portions of the older trees. The tracheid length increased from the stump level towards the top of the tree, at least for the first few feet.

11. Torquimural tracheids are defined as cells with or without interstitial spaces, moderately to very dense, the secondary thickening of the individual cell wall being sharply differentiated from the primary wall by its radial bands or laminae which lie in a spiral around the cell. Torquimural tracheids

were not confined to the leaning side of the tree, but they occurred also on opposite sides of the same cross-section.

12. Failure in compression was accompanied by buckling of the tracheids in the direction of a tangent to the annual rings. The buckling cells separated from each other at the interfaces of the middle lamella and the secondary thickenings. In the concentrimural cells, buckling was accompanied by a folding of the wall on the inner side of the curve, whereas in the torquimural cells, when buckling occurred, it was usually accompanied by a shredding of the laminae, although the tracheids often settled by folding upon themselves or broke off short.

13. The compression strength was roughly proportional to the specific gravity, but there were variations of from 40 to 70 per cent in strength (based upon the minimum strength) for a given specific gravity. Due to the numerous factors affecting strength, the strength-density relationship is better expressed by a band than a straight line. The strength decreased from a maximum in the lower 12 to 16 feet of the tree towards the crown regardless of species. It increased from the region of the pith towards the periphery of the stem. The strength reached a maximum in the outer sapwood of the younger *Pinus Taeda* trees, and from 7 to 11 inches from the pith in the older *Pinus Taeda* tree after which it declined rapidly to a minimum in the outer sapwood, whereas it decreased from the first specimens around the pith towards the periphery of the stem in the *Pinus echinata* and *Pinus palustris* trees.

14. For the low-density material, *Pinus Taeda* had a greater strength per unit weight, whereas for the high-density material *Pinus palustris* and *Pinus echinata* were superior.

15. The strongest wood per unit weight contained straight concentrimural tracheids with few interstitial spaces, whereas wood which was very weak in relation to its density was associated with crooked and torquimural tracheids. The accumulative effects of numerous wood rays and resin ducts also contributed to the weakness of the material.

16. Since the material containing extremely crooked fiber and the more pronounced torquimural tracheids can be dif-

ferentiated in the dressed condition from the better grade of lumber, the above data will aid the man of industry in selecting timbers for special designs. More data of this type will facilitate the formulation of grading rules and make it possible for lumber companies to standardize their products.

The writer wishes to express his gratitude to the officials of the American Creosoting Co., who have made this investigation possible by establishing a research fellowship in the graduate laboratory of the Henry Shaw School of Botany of Washington University and furnished the material for the experiments, and to Dr. Hermann von Schrenk, Pathologist to the Missouri Botanical Garden, for his suggestions and criticisms during the progress of this work and the preparation of the manuscript. The writer is also indebted to Dr. E. S. Reynolds, Physiologist to the Henry Shaw School of Botany, for his suggestions in carrying out the microscopic studies, to Prof. A. W. Brust, of the civil engineering department of Washington University, for help in interpretating the strength data, and to Dr. George T. Moore, Director of the Missouri Botanical Garden, for courtesy extended him in the use of the Garden library. Valuable assistance was rendered by research fellows, Mr. Charles O. Quade and Mr. Chester Abbey (American Creosoting Co. Fellows), in making tests and calculations.

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Explanation of Plate

PLATE 9

Three test specimens from each of the seven trees showing some of the variations in growth rate and percentage of summer wood.

Fig. 1. From tree 1 (*Pinus Taeda*, loblolly pine), showing the extreme range in percentage of summer wood. Specimen 1aN7 at the extreme left is composed almost entirely of torquimural tracheids.

Fig. 2. From tree 2 (*Pinus Taeda*, loblolly pine), showing the great variation in growth rate and the poor differentiation of spring and summer wood in specimen 2hS1 at the extreme right.

Fig. 3. From tree 5 (*Pinus Taeda*, loblolly pine), showing the differences in growth rate and percentage of summer wood in the immediate vicinity of the pith (specimen 5iS1), the intermediate zone (5bN12), and in the outer sapwood (5bN3–5). Note the V-shaped markings in 5bN12.

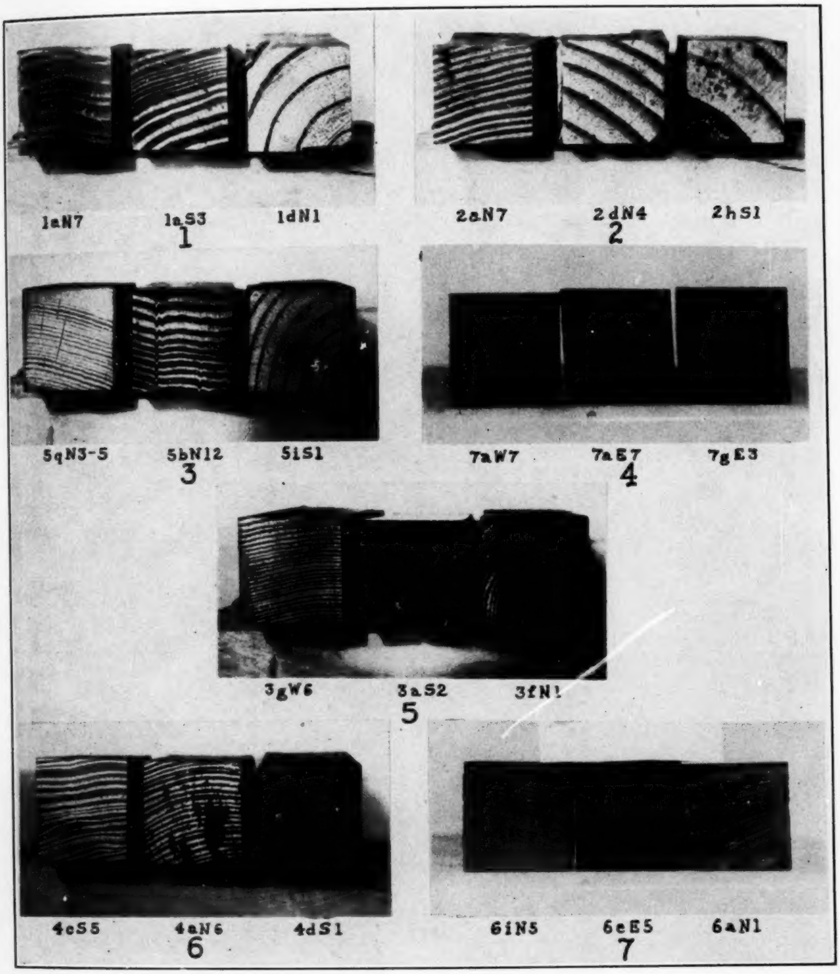
Fig. 4. From tree 7 (*Pinus Taeda*, loblolly pine), showing the major variations in growth rate and percentage of summer wood. Specimen 7gE3 on the extreme right has poor differentiation of spring and summer wood and contains a certain amount of torquimural tracheids.

Fig. 5. From tree 3 (*Pinus echinata*, shortleaf pine), showing the differences in percentage of summer wood and growth rate in the immediate vicinity of the pith at the different heights in the tree as shown in 3aS2 and 3fN1.

Fig. 6. From tree 4 (*Pinus palustris*, longleaf pine), showing variations in growth rate and the irregular rings containing torquimural tracheids as seen in 4cS5.

Fig. 7. From tree 6 (*Pinus palustris*, longleaf pine), showing variations in growth rate and percentage of summer wood. Note the broad rings of summer wood in 6eE5 which are composed largely of torquimural tracheids.

Specimen 1aN7 of fig. 1 is representative of the material found in the weakest specimens per unit weight of the seasoned material, and 4cS5 of fig. 6, of the green material.



BERKLEY—SOUTHERN PINE

EXPLANATION OF PLATE

PLATE 10

Fig. 1. A cross-section of the spring-wood portion of an annual ring showing the distribution of the resin ducts. × 13.

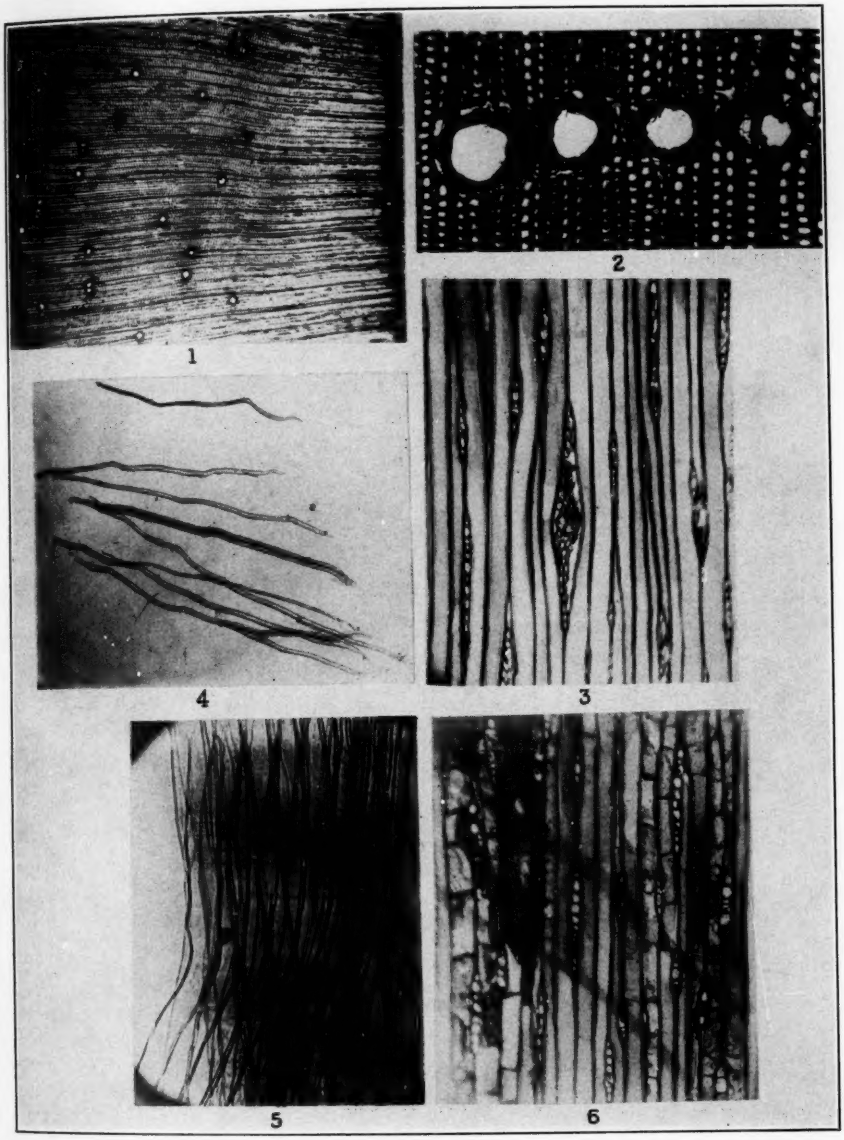
Fig. 2. A row of 4 resin ducts. Note the resin duct on the extreme right branching to form the fifth one. The cells between these resin ducts are all thin-walled parenchyma cells. × 100.

Fig. 3. Concentrimural tracheids showing the curvature and reduction in diameter where they pass the larger wood rays, particularly the fusiform ray. × 100.

Fig. 4. Crooked and deformed tracheids. × 13.

Fig. 5. The crossing of the alternate tiers of tracheids in crooked-fibered material. × 100.

Fig. 6. This figure gives some idea of the extent of the wood parenchyma and the septate tracheids in the region of a series of resin ducts as shown in fig. 2. × 100.



BERKLEY—SOUTHERN PINE

Explanation of Plate

PLATE 11

Fig. 1. The diffraction patterns of the concentric laminae in the cell walls as revealed by the Spierer lens. These are typical concentrimural tracheids. × 900.

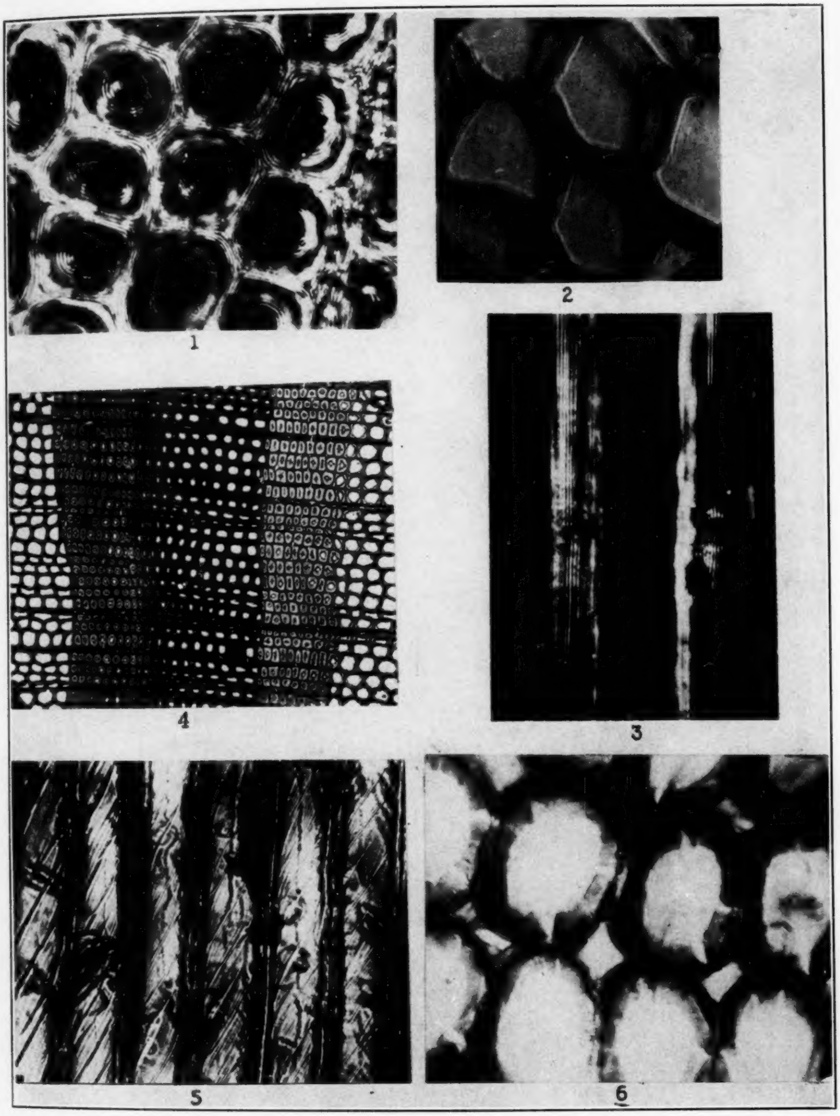
Fig. 2. The concentric laminae of the spring wood cells under direct illumination. This photograph was taken by the use of an ordinary oil-immersion lens. × 900.

Fig. 3. The diffraction patterns of the laminae as shown in the longitudinal section with the Spierer lens. The focus was adjusted on the cell walls and the lumen appears as a dark band between them. × 900.

Fig. 4. A layer of torquimural tracheids (the darkest area) within the summerwood portion of an annual ring. Note the sharp demarcation between the concentrimural cells and the torquimural cells at the inner edge of the latter (to the right) and the gradual disappearance of the torquimural cells on the outer side (to the left) where the two types of cells are intermixed. × 100.

Fig. 5. A longitudinal section of torquimural tracheids showing the spiral checks. Note the striations between the checks. × 450.

Fig. 6. A cross-section of torquimural tracheids showing the interstitial spaces, the sharp demarcation between the primary and secondary walls, and the numerous radial checks in the latter. × 900.



BERKLEY—SOUTHERN PINE

EXPLANATION OF PLATE

PLATE 12

Fig. 1. A cross-section of torquimural tracheids at the right and concentrimural tracheids at the extreme left. The radial striations in the secondary thickening are layers corresponding to the laminae of the concentrimural cells as illustrated in pl. 11, fig. 1. The cells to the extreme left do not have the radial laminae. Photograph taken by the aid of an ordinary oil-immersion lens. × 900.

Fig. 2. The torquimural cells as seen under the Spierer lens. Note the irregularity of the radial laminae. × 900.

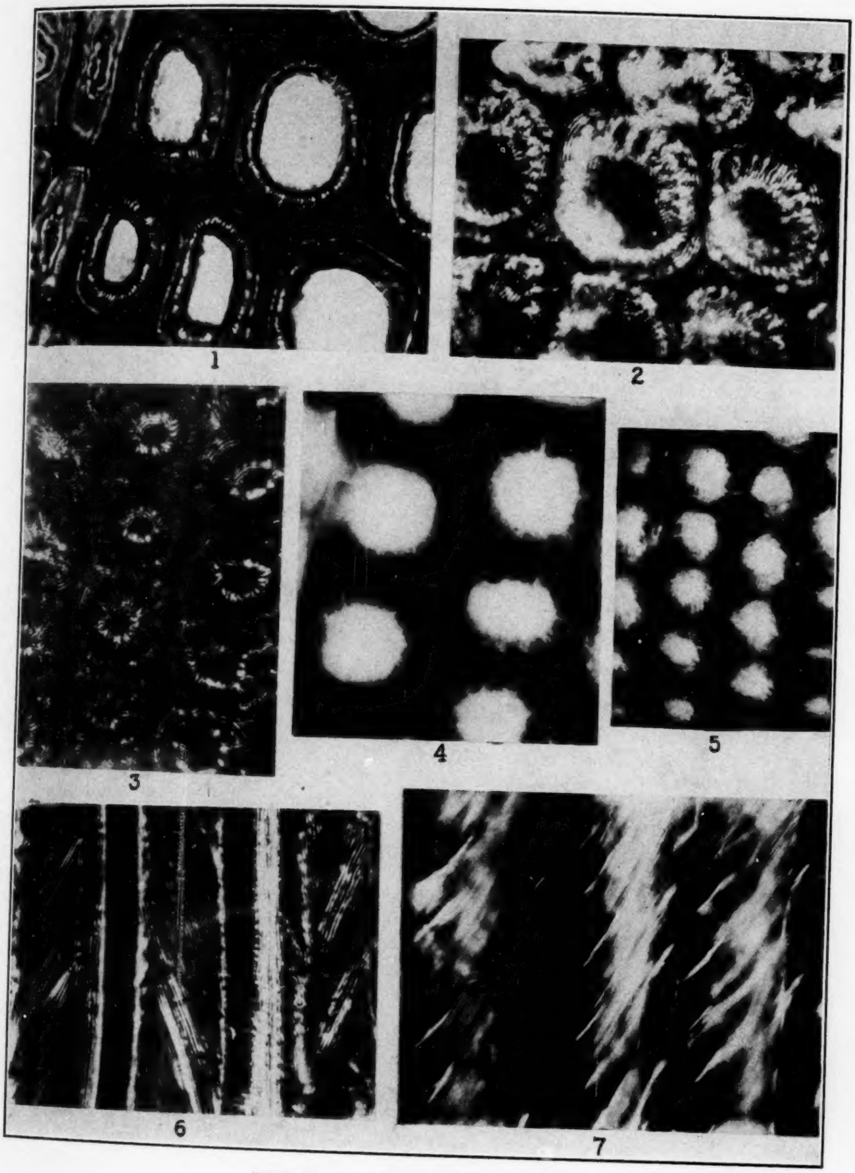
Fig. 3. Torquimural cells as in fig. 2 but showing in addition to the radial laminae of the secondary thickening the concentric laminae of the middle lamella. This area appears somewhat broader than it really is, due to the magnification of the diffraction patterns. × 900.

Fig. 4. The torquimural cells as seen by the use of the cardeoid dark-field condenser. Note the middle lamella which is distinct from the secondary walls which are checked in the radial direction. × 900.

Fig. 5. The same as fig. 4 but at lower magnification which shows more clearly the radial arrangement of the laminae in the secondary thickening. × 450.

Fig. 6. A longitudinal section of torquimural cells as seen under the Spierer lens. Note the spiral arrangement of the striations in the secondary thickening as contrasted with the parallel striations in the region of the middle lamella. This is a tangential section of the cell cutting through the radial laminae of the secondary thickening showing their edge view. Note the area between the middle lamella and the spiral laminae which shows no particular structure. This is due to the angle at which the laminae were cut, showing them on a side view as will be seen if this section is compared with a tangent to the cells shown in figs. 1–5. × 900.

Fig. 7. The spiral striations in the torquimural cells as shown under the dark field. × 900.



BERKLEY—SOUTHERN PINE

Explanation of Plate

PLATE 13

Fig. 1. Typical compression failures. In *a*, two offsets or planes of failure occurred parallel to each other. In *b*, a wedge split resulted where two planes of failure intersected. In *c*, two offsets met near the edge of the specimen. (The fungous growth on *c* occurred after the specimen was tested.)

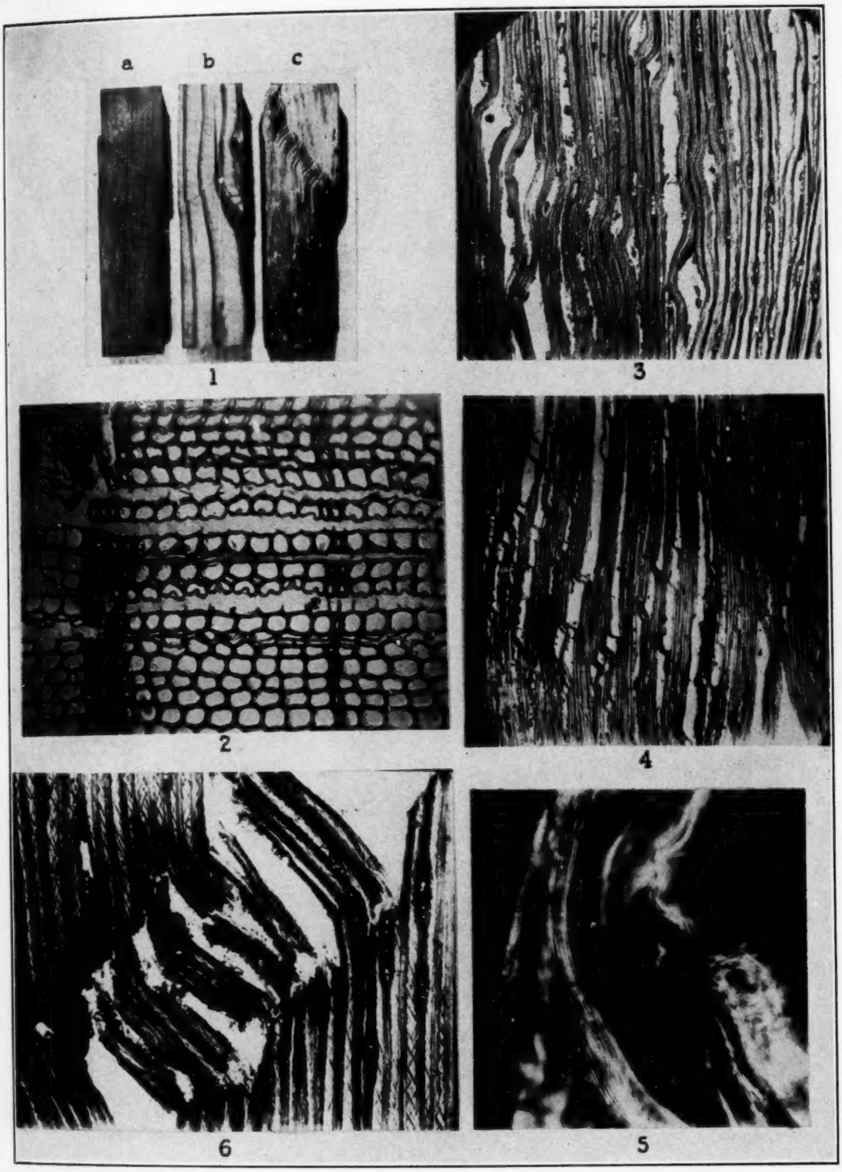
Fig. 2. A cross-section through the zone of failure, showing the tiers of tracheids separated from each other. Note the spring-wood cell walls folded into the lumina of the cells and the fragments of the middle lamella pulled from between. These folded walls represent only the secondary thickening since the middle lamella was still attached to the opposite cells. × 100.

Fig. 3. Tangential section through the region of failure showing buckled tracheids. Note the openings at the rays and the tendency of certain tracheids to buckle in opposite directions at different points due to the curvature of the cells and the direction of the major failure. × 100.

Fig. 4. A region of failure showing the extent of the failure in regions which are not visible to the unaided eye. × 50.

Fig. 5. A short segment of the buckled portion of a cell as seen under the Spierer lens. Note the folding of the inner wall. × 900.

Fig. 6. A brash failure typical of seasoned torquimural cells. Note the cross hatching in the cell walls due to the checks which tend to show through from the opposite walls, thus appearing to cross. × 100.



BERKLEY—SOUTHERN PINE

PORIA COCOS (SCHW.) WOLF, FOUND ON A RAILROAD TIE IN SERVICE

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The tuckahoe was identified as *Poria Cocos* by Wolf,[2] who was the first to find the mature fructifications associated with the sclerotium. The range of hosts and the synonymy for this organism have recently been summarized by Weber.[3] For the most part it has been found associated with the roots of trees as a parasite. Ravenel,[4] however, found a number of small specimens partially embedded in the decayed wood of an old pine rail, but no rhizomorphs or other direct attachments were observed.

The specimen shown in pl. 14, fig. 1, was found by workmen on the Memphis Terminal of the Missouri Pacific Railroad on a *Taxodium* cross-tie which they were removing from the tracks, and it was given to the writer by Dr. Hermann von Schrenk.[5] The sclerotium was attached to a lower corner of the tie about midway between the tracks and was concealed by cinder ballast. The track at this point was on a fill where it was usually dry. The tie had been in service about eight years and was badly decayed.

The dark brown rind of the sclerotium, which was sharply contrasted with the interior, was corrugated and resembled somewhat the bark of a tree. This rind and a few millimeters of the area beneath it contained fragments of the host tissue,

[1] A fellowship established by the American Creosoting Co.

[2] Wolf, F. A. The fruiting stage of the tuckahoe, *Pachyma Cocos*. Elisha Mitchell Scientif. Soc., Jour. **38**: 127–137. 1922.

[3] Weber, G. F. The occurrence of tuckahoes and *Poria Cocos* in Florida. Mycologia **21**: 113–130. 1929.

[4] Ravenel, H. W. Note on the tuckahoe. Bull. Torr. Bot. Club **9**: 140. 1882.

[5] The field notes were furnished by Mr. K. G. Williams, Division Engineer at Memphis, Tenn. (letter of G. R. Westcott, dated Nov. 23, 1933, in files of Dr. Hermann von Schrenk, file 16214).

Issued June 5, 1934.

and the wood to which the tuckahoe was attached was thoroughly permeated by the fungous hyphae. The lumina of the tracheids and the intercellular spaces were completely filled with the globular masses of mycelia, and layers of it in the form of pads were interspersed with the tiers of tracheids which split the wood radially. The interior of the sclerotium was similar in all respects to the dry specimens described by Wolf[6]; it was white to cream in color, hard and bony, with many large cracks. When boiled in water for some time, this material became soft and doughy.

On the surface of the sclerotium and on the wood adjacent to it there were a number of fructifications containing mature basidiospores. These fructifications resembled in all details those described by Wolf[6] and Weber.[6] The basidiospores were white, asymmetrically cylindrical, and about 3.5–4.0 x 7.5–8.0 μ in size. Additional fructifications were obtained within five or six days by placing portions of the sclerotium, which had been soaked in water, in a moist chamber in the laboratory.

On the surface of the sclerotium in a hymenial layer, numerous dark brown conidia were found. These were smooth, obovate, and about 3–4 x 5–6 μ in size. They were for the most part in the depressions of the corrugated surface of the sclerotium, and those observed were terminal. There was no attempt made to germinate them.

The discovery of this specimen on a *Taxodium* cross-tie, together with those reported by Ravenel[6] on a pine rail, indicates that the fungus is not entirely parasitic on roots or stems of living plants but may be saprophytic on wood as well. It may be considered then as a facultative parasite.

[6] *Loc. cit.*

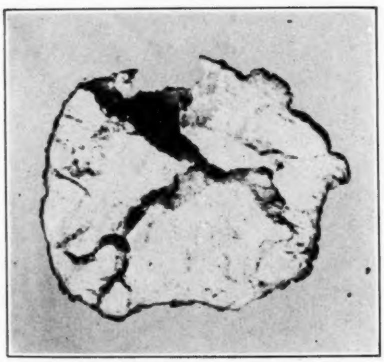
EXPLANATION OF PLATE

PLATE 14

Fig. 1. A photograph of a tuckahoe *Poria Cocos* (Schw.) Wolf. The white spots are the fructifications.

Fig. 2. A section of the tuckahoe in fig. 1, showing the thin rind and white interior. The interior cracked badly on drying but the surface remained intact.

1



2

BERKLEY—PORIA COCOS

FIELD AND HERBARIUM STUDIES, III[1]

LOUIS WILLIAMS

Washington University Fellow in Botany

The first in this series of short papers dealing with the plants of the Rocky Mountains appeared in the Bulletin of the Torrey Botanical Club **59**: 427-429. 1932; the second, in the same journal **61**: 259-262. 1934.

Corallorrhiza striata Lindl. var. **Vreelandii** (Rydb.) comb. nov.

C. Vreelandii Rydberg in Bull. Torr. Bot. Club **28**: 271. 1901.

Malaxis Soulei, nom. nov.

Microstylis montana Rothrock, Rept. Bot. Wheeler Exp. 264. 1878.

Malaxis montana Kuntze, Rev. Gen. Pl. **2**: 673. 1891, not Blume. 1826.

Acroanthes montana Greene in Pittonia **2**: 183. 1891.

Polygonum minutissimum sp. nov. Annuum perparvum glabrum, 0.5–1 cm. altum; folio uno ad basem caulis, lineare, acuto; inflorescentia axillare, cum 4–10 floribus; sepalis albis, petalinis; achaeniis fuscis, ovatis, 1 mm. longis, triangularibus.

Minute glabrous annual 0.5–1 cm. tall; one leaf at the base of the stem, linear, acute, about equalling or surpassing the inflorescence, sheathed at the base by a hyaline ocrea; inflorescence axillary, 4–10-flowered, each flower subtended by a foliar bract about 2 mm. long; sepals white with a green midrib, petal-like, as long as the achenes; achenes dark brown, 3-angled, ovate, 1 mm. long, styles 3.

Collected in full fruit under overhanging cliff near Hidden Falls, Grand Teton National Park, Wyoming, July 15, 1932, *Williams, 878* (Ry. Mt. Herb. TYPE; Mo. Bot. Gard. Herb., Herb. Phila. Acad. Nat. Sci., Herb. Geo. E. Osterhout, Herb. L. Williams).

[1] Issued June 5, 1934.

This species belongs in the section Avicularia, but is not closely related to any species known to the author.

Aquilegia Piersoniana sp. nov. Perennis alpestris humilis ex rhizoma crassa, 10–30 cm. alta; foliis biternatis, segmentis cuneatis, 3-lobatis, glabris, subtus glaucis; floribus flavis, 20–25 mm. longis, nutantibus; sepalis oblongo-ovatis, breviter unguiculatis, 2 cm. longis, non reflexis; laminis 6–7 mm. longis, patulis; calcare gracili, 12–14 mm. longo.

Low slender alpine perennial from a thick root-stock, 10–30 cm. tall, tufted or single; stems pubescent above, otherwise glabrous; leaves biternate, the largest leaflet 2–2.5 cm. broad, the segments of the leaflets cuneate, about 1 cm. long, 3-lobed for about one-third their length, glabrous, slightly glaucous beneath, the basal leaves half or more the length of the stem; stem-leaves much reduced, sessile, 3-parted, the lobes oblong; infloresence one–several-flowered, flowers nodding, yellow or occasionally tinged with blue; sepals oblong-ovate, short-clawed, 2 cm. long including the claw, 1 cm. wide, not reflexed, dorsally pubescent, blade 6–7 mm. long, spreading; spur slender, not enlarged at the end nor reflexed, 12–14 mm. long, slightly pubescent; capsule unknown.

Collected on wet ledges on the north base of the Grand Teton, altitude about 11,000 feet, Grand Teton National Park, Wyoming, August 10, 1932, *Williams, without number* (Ry. Mt. Herb. TYPE). Named in honor of Miss Rua Pierson in recognition of her interest in the local flora.

This species is perhaps most closely related to *A. flavescens* Wats. from which it differs in the following characters: The sepals and petals are both longer than the spurs, and neither seem to be reflexed; the spurs lack the enlarged tips and are more slender; the flowers are apparently erect in the bud and are much shorter.

Hedysarum pabulare A. Nels. var. **rivulare** var. nov. A specie foliis longioribus, angustioribus obtusioribusque, supra glabris, subtus pubescentioribus differt.

Collected on rocky flats along Snake River, Bar BC Ranch, Teton Co., Wyoming, July 31, 1932, *Williams, 975* (Ry. Mt.

Herb. TYPE; Mo. Bot. Gard. Herb., N. Y. Bot. Gard. Herb., Herb. Catholic Univ. Am., Herb. Geo. E. Osterhout., Herb. Calif. Acad. Sci., Herb. Utah Agr. Coll., Herb. Our Lady of the Lake Coll., Herb. L. Williams).

Penstemon acaulis sp. nov. Perennis depressa acaulis, 2 cm. vel minus alta; foliis lineari-acutis, 1–2 cm. longis, in corona fasciculis vel verticillis dispositis, puberulo-glandulosis; sepalis 5 mm. longis, ovato-attenuatis; corolla coerulea, in corona radicis, 15 mm. longa, fauce cum crinibus flavis infra; staminibus glabris, filamentis affixis ad vel prope basem corollae, posterioribus brevioribus; loculis antherae confluentibus; stamine sterile filiforme, crinibus fulvis ad altero latere ⅔ longitudinis tecto.

A depressed acaulescent perennial 2 cm. or less high, caespitose; roots slender; leaves linear-acute, 1–2 cm. long, 1 mm. wide, widest near the more or less calloused tip, usually borne in bunches or whorls on the crown or short proliferous branches; sepals 5 mm. long, ovate-attenuate, minutely glandular-puberulent; corolla blue, about 15 mm. long, usually subtended by a few leaves, much expanded above, 6–8 mm. wide at the throat, more or less glandular on the outer surface, the lobes rounded, 3 mm. long, more or less conduplicate, yellow-hairy in the throat, mostly on the middle lobe; stamens glabrous, the posterior pair reaching the throat, the anterior pair attached at the base and shorter than the posterior which are attached one-third the way up the corolla-tube, filaments filiform, anther-cells confluent; sterile stamen filiform, covered with crisp golden-brown hairs on one side for two-thirds of its length; stigma not enlarged; capsule unknown.

Collected in flower on dry hilltops near McKinnon, Sweetwater Co., Wyoming, altitude about 6,500 feet, May 28, 1932, *Williams, 407* (Ry. Mt. Herb. TYPE; Herb. Phila. Acad. Nat. Sci., Herb. N. Y. Bot. Gard., Mo. Bot. Gard. Herb., Herb. Geo. E. Osterhout., Herb. Calif. Acad. Sci., Herb. Catholic Univ. Am., Herb. Our Lady of the Lake Coll., Herb. Utah Agr. Coll., Herb. L. Williams).

This species belongs in the section Caespitosi (Pennell,

Contr. U. S. Nat. Herb. **20**: 334. 1920). From the species recorded in that treatment it may be easily distinguished by the strongly cespitose habit and apparent lack of stem. Thanks are due Dr. Francis W. Pennell, who has given his opinion of the plant. He says it "is the most condensed in habit of any of that genus" and "differs from all others in lacking any definite stem."

POSADASIA PYRIFORMIS AND P. CAPSULATA, TWO CAUSATIVE ORGANISMS OF DARLING'S HISTOPLASMOSIS IN THE UNITED STATES[1]

MORRIS MOORE

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As far as the author could determine, there has been no satisfactory classification of the fungi responsible for the condition known as Darling's Histoplasmosis. This disease is characterized by an acute specific infection involving usually the epithelial and endothelial cells of the lungs, liver and spleen. The organism may also be free in these organs, as well as in the blood stream. The following are two organisms from such a condition.

Posadasia pyriformis Moore, n. sp.

In the host reproduction by single yeast-like cells. On artificial media, mycelium of septate, elongate or short, thick hyphae, 1–5 μ in diameter. Macroscopically, cultures cottony with aerial hyphae, hyaline and white in a mass to a dark Isabella in color, with a diameter of approximately 2–6 cm. on various media, after 43 days' growth. Microscopically, many conidia, lateral, sessile or pedicellate, spherical or pyriform, 3–8 μ in diameter; chlamydospores, intercalary 3–10 μ in diameter, singly or in chains and lateral on 1- to several-celled branches, or terminal 3–10 x 6–20 μ; racquet mycelium present; sexuality lacking; clavate or globose cells 5–18 μ in diameter, becoming multispored tuberculate asci, spherical 6–25 μ in diameter, usually 15 μ, and pyriform 6–12 x 12–26 μ, usually 10 x 22 μ. Tubercles up to 7 μ in long axis, varying in number and proportions. Carbohydrates not fermented. Milk not curdled or acidified. Gelatine not liquefied.

Posadasia pyriformis Moore, sp. nov.

Mycelium in culturis abundans sed in hospite cellulae singulae sunt. Hyphae longae vel breves, diametro 1–5 μ. Cul-

[1] Issued June 5, 1934.

turae floccosae aeriaeque, diametro 2–6 cm. diversis in mediis postquam 43 diebus. Conidia multa lateralia sessilia vel pedicellata, spherica vel piriformia, diametro 3–8 µ; chlamydosporae intercalarae lateralesve diametro 3–10 µ vel terminales 3–10 x 6–20 µ; mycelium "racquet" adest. Sexus deest. Cellulae clavatae vel globosae, diametro 5–18 µ, ascos multisporos tuberculatos fiunt, diametro 6–25 µ vel piriformes 6–12 x 12–26 µ. Tuberculi longi, 1–7 µ, numero et magnitudine diversi sunt. Gelatinum non fluidificans. Lac non concretum, acidus nullus. Fermentatio nulla.

Posadasia capsulata (Darling) Moore, n. comb.

Histoplasma capsulatum Darling, Jour. Am. Med. Assoc. 46: 1283–1285. 1906.

This species differs from *P. pyriformis* in having a slightly smaller growth on corresponding media and a light Isabella color. Microscopically the cells are smaller in proportion; hyphae 1–4 µ; conidia 2–7 µ; chlamydospores spherical, 3–8 µ in diameter, pyriform, 3–9 x 7–18 µ; clavate or globose cells 5–15 µ; asci multispored, spherical, only 5–22 µ, rarely 25 µ. Sexual development absent. Gelatine not liquefied. Milk not curdled or acidified. Carbohydrates not fermented.

Complete morphological, cultural, biochemical, and cytological details will follow.

A NEW GEOTRICHUM FROM A BRONCHIAL AND PULMONARY INFECTION, GEOTRICHUM VERSIFORME MOORE, N. SP.[1]

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Introduction

The purpose of this paper is to report the occurrence of a case of a bronchial and pulmonary infection from St. Louis, Missouri, and also to present the characteristics of a *Geotrichum* isolated from it. The organism is described as a new species, and from all indications it was the probable etiologic agent of the disease.

The genera *Geotrichum, Mycoderma, Oidium, Oospora, Monilia,* and several others have been confused by numerous authors. Nomenclature has been changed, terms have been discarded and others have sprung up, but apparently no universal agreement has been reached by all interested mycologists. The author wishes to indicate some important facts as to the structure and differentiation of the fungus, as well as to point out apparent misnomers and the correct position of such incorrectly determined organisms.

Case Report

Clinical History.—Barnes Hospital Clinic No. C 17521. Patient F. S., a white male, 22 years of age, a chemist, entered the clinic March 9, 1931, for some ailment to be shown later, and was then released. He re-entered December 31, 1932, with a persistent, productive cough which brought up a thick, greenish, muco-purulent, tenacious sputum.

Family History.—Mother died in childbirth 15 years previously. Father living and well. One brother living and well.

[1] A paper reported at the meetings of the Mycological Society of America, on December 30, 1933, held in conjunction with the ninety-third meeting of the American Association for the Advancement of Science, at Boston, Massachusetts, December 27, 1933–January 2, 1934.

Issued June 5, 1934.

Past History.—Whooping cough when child. Pneumonia 6 years previously; in bed 2 weeks and no sequelae. Measles in January, 1931, followed by a mastoiditis. Mastoidectomy performed at the Barnes Hospital in February, 1931. Two or three attacks of tonsillitis since 14 years of age, with abscess at one time. Tonsillectomy performed at the Barnes Hospital, June 6, 1931.

Personal History.—Wassermann and Kahn negative.

Present Illness.—About 7 years previously, while a student in high school, the patient kept irregular hours and began to have a more or less persistent cough, on one occasion the sputum being bloody. Cough continued for several years, usually worse in the morning, and about one to two tablespoonsful of sputum, occasionally bloody, was coughed up. The summer before entry, patient was in the open a great deal and the cough subsided. About a month previous to entry, he developed a fever with generalized pains. He was confined to his bed and since that time the cough became more persistent and painful and troubled him mostly at night.

A chest examination revealed a few squeaks. Fluoroscopic examination showed little. In view of the history of chronic cough, sputum examination was advised and X-ray with flat plate on sternum.

January 4, 1933.—To date the presence of an infection or dangerous condition not indicated. Further examination to be carried out.

January 9, 1933.—Patient fairly well, but complained of persistent cough in morning and occasionally a coughing spell in the afternoon, with which a large amount of pale yellow, occasionally streaked sputum was brought up. Physical findings of lungs showed some roughening on both sides and some increase in left and right lower lungs, with râles.

Diagnosis.—Bronchiectasis. Lipoidal suggested.

January 10, 1933.—Lipoidal introduced into left lung. X-ray showed definite bronchiectasic areas or multiple abscesses. During course of introduction, patient coughed up about 60 cc. purulent material containing bright blood streaks.

January 14, 1933.—Patient returned to the chest clinic. He stated that he had been growing mushrooms for the past year in an underground quarry where the humidity was fairly high. Felt fairly well at the time, but still brought up a moderate amount of sputum. Further examination of sputum for molds.

January 28, 1933.—Definite bronchiectasis of base of left lung. Posterial drainage.

X-ray findings of January 3, 1933.—Posterior-anterior view of chest. Cardiac shadow within normal limits. Tremendous increase of shadow of left hilus and generalized thickening of lung markings throughout the field. Particular widening of those markings lateral to the right cardiac border at the base, associated with a large amount of coarse, soft, slightly coalescent mottling. Cloudiness within the circle of the first rib on either side, at the extreme apex. Leaves of diaphragm rounded and the costophrenic angles clear.

X-ray Diagnosis.—Pulmonary infiltration of left base, indeterminate nature.

The sputum was cultured and the organism described in this paper was isolated in relatively great amounts. The patient was given potassium iodide *per os*. He left St. Louis sometime during the spring of the year 1933, and in a letter to the author, dated June 23, 1933, he stated that his general health had improved, with a gain of five pounds in weight. The pain in the chest had disappeared except for

infrequent periods, the cough was somewhat better, and the amount of sputum had decreased. He had stopped treatment about a month previously. A second communication dated October 26, 1933, stated that his condition had not changed, that he had resumed the previous treatment for a short time but upon finding no marked improvement had again stopped it.

Technique

The fungus was studied in hanging-drop preparations or Van Tieghem cells, in a lactose-broth medium, a product of the Digestive Ferments Co., as well as on Sabouraud's broth, 2 per cent bacto-peptone, and meat extract broth.

For morphological detail and for possible cellular granulations, the fungus was mounted in a 1 per cent aqueous crystal violet solution plus glycerine, the desired amount of dye being added to obtain the necessary intensity. Amann's lacto-phenol was also used, as well as various dyes in aqueous solutions, but the first two were sufficient for all purposes of general structure. Distilled water mounts were made which showed up the oil globules present on the cellular surface.

Description

Geotrichum versiforme macroscopically assumes different conditions, varying from crinkled and vermiculate on malt extract agar to asteroid on Sabouraud's agar. The velvety or plush-like appearance prevails on most culture media, conspicuously so on potato-dextrose and glycerine agar, and many of the cultures, particularly those on meat extract, lactose, and Endo's agar, are moist. On Raulin's and Richards' agar, which contain inorganic sources of nitrogen and several salts, the mycelium is completely submerged. The colonies are tenacious and hard to separate from the substrate. The striations evident on Sabouraud's agar are due to coremia formed by the matting of hyphae. On liquid media, a pellicle is formed on the surface with a fine sediment in the bottom of the flask.

When isolated from the sputum, the fungus is in the form of simple cells, ovoid to spherical, 4–6 μ in diameter. When grown on agar, the cells form a complicated mycelium which eventually breaks up into arthrospores typical of this group. On artificial substrates, the arthrospores serve as the seeds of

the future colonies. The various steps in their formation may be traced as follows: The single cell germinates, sending out a thin-walled tube which may emerge from either end, or laterally (pl. 16, figs. 1–6). The germ-tube may be simple (figs. 7–8) or it may bi- or trifurcate. It elongates and forms cross-walls which may be simple or collar-like (figs. 7–8, 15), either equidistant or irregularly spaced, when secondary cross-walls may develop (fig. 17). With the development of equidistant partitions, the hypha is now divided into a number of cells. Langeron and Talice ('32) observed that the germ-tube may remain simple and undivided, as stated previously, and as such will bifurcate or trifurcate. This was occasionally noted here, but these germ-tubes later became arthrospores. It is to be noted that the hyphae are capable of developing lateral branches (figs. 12, 14–17), which may form simply or as off-shoots at or below a cross-wall. In addition, the so-called blastospores may be formed as suggested in fig. 12.

The presence of a true conidium in *Geotrichum* seems to be much disputed. One cannot help feeling, however, that a pyriform structure such as that in pl. 16, figs. 11, 14, and 16, having the functions of a conidium as noted in other organisms, e. g. *Endomyces*, are very highly suggestive of that organ. There is no doubt that the cells are capable of germinating into a tube which has the same vegetative and reproductive functions as the arthrospores. That being the case, the only reason why that particular cell may not be called a conidium is cytological, and such a study will be reported later.

The young filaments are thin-walled at first, and as the cross-walls are laid down, the thick hyaline walls cause the resulting cells to assume a double-contoured appearance. Just previous to disarticulation, or the breaking up of the filament into the arthrospores, the cells may assume a rectangular appearance (pl. 16, fig. 18), or they may be barrel-shaped (fig. 23). On various media and hydrogen-ion concentrations they may assume different forms, as on malt extract agar (fig. 19) and on Richards' agar (figs. 24, 28) where they are in the form of oidia. It must also be noticed that not all hyphae develop these equidistant cells, for irregularity is likewise well-marked (figs.

19, 22). Also, the smaller cells may be formed only on a portion of the filament, as seen in figs. 27, 30, 32, a condition which is apparent in the Oosporaceae of Saccardo, particularly as seen in fig. 27.

When mature, the thick-walled cells become arthrospores. A gelified secretion seems to be laid down simultaneously between the cells as part of the connecting cell-wall, at the point where disarticulation takes place. The arthrospores appear at first to be cylindrical (rectangular in optical section) (pl. 16, figs. 18, 23), and in many cases (figs. 22, 24) are rounded at the ends. When completely separated from each other, the cylindrical arthrospores become spheroidal, ovoid, or ellipsoid (fig. 26). In a few cases, it is possible to note some of the protoplasm connecting the arthrospores, which has not completely attached itself to the cells, or perhaps part of the cell-wall which has not entered into the formation of the cells, as in fig. 18. The cells may then either remain in the condition described above or they may become spherical, ovoid, or even ellipsoid, and within a suitable period of time germinate to give rise to a new colony.

Several other anatomical structures have been given consideration by various workers and should be noted here. First, not all the hyphae or even a whole filament will divide to form arthrospores. Disarticulation may occur either near the end of a filament as in pl. 16, figs. 27 and 30, or at various segments of a filament. In either case, the intervening portion of the hypha is clear and thin-walled and seems to disintegrate after the arthrospores have been set free. Apparently the cellular material is used up in the formation of the thick-walled cells. On a few occasions, arthrospores or perhaps chlamydospores were found enveloped by a thin membrane (fig. 32), the entire wall evidently not having entered into the development of the spores.

Chlamydospores are found frequently. These are distinguished from the rest of the mycelium by their conspicuous size and seemingly granular protoplasm (pl. 16, figs. 13, 19–20, 23, 33), and in some instances (fig. 33), they simulate the akinetes as found in algae. Cells analogous to terminal chla-

mydospores (hypnospores) are also evident (figs. 29, 31, 34). Chains of oidia-like cells on Richards' agar (fig. 28) are of common occurrence on that medium, and evident also on Raulin's solution agar. Occasionally blastospores occur, either thick- or thin-walled (perhaps suggested by fig. 22).

In addition to the various organs discussed above, the mycelium may be altered, changes in the constituents of the substrate giving rise to numerous structures indicative of standard fungus organs as well as nondescript, sclerotic cells and the racquet mycelium characteristic of various other groups of fungi.

Cultural Descriptions

The culture obtained in this study was growing on a Sabouraud's glucose-agar slant. The colony assumed a velvety "duvet" appearance, with lines radiating to the periphery, apparently a coremioid condition. Transfers were made to various media which ranged in pH from 4.1 to 7.5, also to more strongly alkaline and more strongly acid media. All cultures were grown at approximately 25° C.

The importance of physiological variations in taxonomic differentiation cannot be stressed sufficiently. Species differentiation founded on growth on a single medium is usually unreliable, and the use of standard media is essential for obtaining the morphological changes. The characteristics here discussed were observed from the growth of the organism on several media which represent a series varying in hydrogen ion concentration, protein and carbohydrate content. The media are arranged in the order of their decreasing concentration of hydrogen ions. The cultures were examined 3–8 days after inoculation and again 210 days later.

Raulin's Solution Agar (pH 4.1).—(pl. 16, figs. 1, 18, 26, 29). Colony dull gray in color, turning faintly cream with age, and attaining a diameter of 6.5 cm. 8 days after inoculation. Smooth and velvety, with a peripheral zone of fine radiate growth. Young culture shows many fine hyphae 2–3 µ in diameter, which develop a great number of cylindrical cells approximately 4 µ in diameter and 7–10 µ long, several round cells ap-

proximately 6–15 μ in diameter, large cells 6 x 15 μ. Short chains of round cells approximately 6 μ in diameter. Chains of arthrospores numerous.

Richards' Solution Agar (pH 4.3).—(pl. 16, figs. 2, 22, 24, 28). Colony of submerged, hyaline mycelium, 5½ cm. in diameter at end of 8 days, with fine radiating lines from the inoculum as seen with the aid of light coming through the agar. Long chains of spherical to rectangular cells 6 x 30–40 μ, large cells varying in diameter from 9 to 21 μ, also variously formed sclerotic cells. Arthrospores and chlamydospores in abundance in older cultures.

Czapek's Agar (pH 4.4).—(pl. 16, figs. 10, 14, 31). Macroscopic appearance similar to that on Richards' agar. Colony 5½ cm. in diameter after 8 days' growth. Long filaments 3 μ in diameter, with enlarged terminal cells 6 x 9 μ. Ovoid cells, arthrospores 6 x 8 μ. Rectangular cells with rounded corners 4 x 6 μ. Elongated cells 3 x 13–15 μ.

Malt Extract Agar (pH 5.2).—(pl. 15, fig. 1; pl. 16, figs. 11, 13, 19–20, 23, 35). Growth irregular and thick in center with a pebbly, vermiculate surface, attaining a diameter of approximately 2½ cm. in 8 days. Colony dull creamy-buff in color, appearing pasty at periphery of colony similar to a yeast culture. Mycelium thick and tenacious, adhering to the substrate and resistant to the needle. Large sclerotic cells, ovoid chlamydospores and arthrospores 20 x 30 μ, and many round cells 15 μ in diameter at center of colony. Variously formed cells and arthrospores varying from 4 to 6 x 6 to 15 μ. Young filaments and yeast-like cells at edge of colony.

Sabouraud's Agar (pH 5.6).—(pl. 15, fig. 2; pl. 16, figs. 3–5, 7, 9, 16, 27, 30). Growth good, 6½ cm. in diameter after 8 days, hyaline to white when young, becoming creamy yellow to light buff when older, with a "duvet" or furry appearance, in sectors, extending from the inoculum. Several colonies showed a moist, shiny, convoluting, cerebriform surface. Mycelium thick with a heavy mucoid tenacity.

With Maltose.—(pl. 16, figs. 27, 30). Round cells approximately 5 μ in diameter, and elongated cells 5 x 8–9 μ. Many thick-walled rounded arthrospores. Young filaments thin-

walled and long, approximately 4–5 x 30–40 µ. Chains of arthrospores numerous in older cultures.

With Glucose.—(pl. 16, figs. 3–5, 7, 9, 16). Cells approximately 3 µ in diameter. Variety of filaments, some branching, long and thin, 2–3 µ in diameter, others short and thick-walled, about 5–6 µ in diameter. Sclerotic cells present, as well as numerous thick-walled arthrospores 5 x 8–10 µ, and chlamydospores (terminal, as hypnospores), pyriform to ovoid and ellipsoid. Lateral cells on a filament similar to conidia, pyriform to round, 6–7 x 5–15 µ. No budding recognizable as such.

Sabouraud's Broth (The above minus the agar, pH 5.6).—(pl. 16, fig. 15). Scum formed on surface of broth with a sediment. Liquid faintly cloudy, becoming clear after a few days. Long filaments with cells approximately 4 x 90 µ. Arthrosporous cells few, 4 x 12 µ. Chlamydospores very few.

Potato-Dextrose Agar (pH 5.9).—(pl. 15, fig. 3; pl. 16, fig. 12). Colony 7 cm. in diameter after 8 days. Cultural appearance similar to that on Sabouraud's agar, with the "duvet," but dull gray to light cream in color. Sectors present showing variability in color, creamy-white as present on Sabouraud's agar. Older cultures plush-like or furry, with a whirl. Long filaments 3 µ in diameter, round cells (chlamydospores) 5–6 µ in diameter; arthrospores numerous, 3–4 x 12–15 µ. Many pyriform loose cells 6 µ in diameter.

Corn-Meal Agar (Product of Digestive Ferments Co., pH 6.0).—(pl. 16, figs. 21, 32–33). Colony 3½ cm. in diameter after 8 days, with distinct zones of decreasing growth from the inoculum to the periphery. Color dull creamy white. Young cultures show long thin-walled hyphae with cells approximately 4–8 x 30–35 µ, many cells varying in same proportions. Large cells show a clear cytoplasm, not taking a stain. Older cultures show an abundance of arthrospores 5–6 x 10–12 µ, as well as large cells of this nature, 9 x 15 µ, round cells 5 µ in diameter. Numerous intercalary chlamydospores, as in fig. 33, simulating akinetes in algae.

Lactose Broth (Product of Digestive Ferments Co., pH 6.8).—(pl. 16, fig. 17). A thin scum or veil on the surface of the

liquid with a macroscopically fine sediment and a clouded condition prevailing throughout for several days. Clusters of hyphae, coremium-like, 4–5 μ in diameter, branching and with cross-walls. Older hyphae, 6 μ in diameter, showing septal formation. Numerous chains of arthrospores 4–6 x 6–11 μ. Ovoid cells 6 x 9 μ.

Lactose Agar (The above plus 2 per cent agar).—(pl. 15, fig. 4). Flat growth of fine filaments, dull gray to light cream in color, with a diameter of 4 cm. after 8 days. Young hyphae 3–4 x 25–35 μ. Older cultures with sclerotic cells and pyriform cells simulating the conidia of *Endomyces capsulatus.* Many large round cells 9–15 μ in diameter, numerous arthrospores 4–5 x 8–11 μ, and many filaments with a terminal club-like appearance.

Glycerine Agar (Beef extract agar plus 6 per cent glycerine, pH 7.0).—(pl. 15, fig. 5; pl. 16, fig. 8). Colony 4.2 cm. in diameter after 8 days, creamy-yellow to light buff in color with a powdery appearance. Center somewhat crateriform, becoming raised, thick and tenacious with age. Slight striations extending from the periphery to the center of the colony. Lateral view of the culture presents a hyaline sheen. Old cultures show numerous arthrospores 3–8 x 8–18 μ; round cells 6–9 μ in diameter; intercalary chlamydospores 6–8 μ in diameter. Filaments or hyphae cross-walled and branching, young filaments with fewer cross-walls and smaller diameter than the older ones. Sclerotic appearance of mycelium similar to that on malt extract agar.

Nutrient Agar (Product of Digestive Ferments Co., pH 7.2).—(pl. 15, fig. 6; pl. 16, figs. 6, 25, 34). Colony moist and flat, cream to light buff in color, with a diameter of 5 cm. in 8 days. Filaments multibranched with racquet-like swellings, a condition prevalent on most cultures and particularly well marked on malt extract and glycerine agar. Hyphae 4 μ in diameter. Older cultures show numerous chains of arthrospores 4–6 x 6–9 μ; round cells 6–12 μ in diameter.

Endo's Agar (Product of Digestive Ferments Co., pH 7.5).—(pl. 15, fig. 7). Cultures flat and pink in color, 3 cm. in diam-

eter after 8 days, moist and shiny with concentric rings of growth and a periphery of fine filaments. Microscopically similar to cultures on nutrient agar.

Gelatine.—After 12 days plain gelatine liquefies slowly on surface at point of inoculation and proceeding downward. Beef extract gelatine (15 per cent) liquefies slowly on surface after 14 days.

Carbohydrate Reactions.—No fermentation on any sugar. Acid and no gas with l-xylose, galactose, d-mannose, levulose, and maltose. No acid, but an alkaline reaction with l-arabinose, rhamnose, dextrose, lactose, sucrose, raffinose, and inulin. Alkalinity may be considered as a negative acidity reaction and may perhaps be accounted for by the breakdown of the protein, amino acids to alkaline bases as arginine, lysine and histidine and finally ammonia, which are the breakdown products in the growth process of the organism.

Litmus Milk.—Acidified and curdled after the fourth day.

Discussion

The genus *Geotrichum* has often been confused with several other genera, e. g. *Mycoderma, Oidium, Oospora* and even *Monilia.* Several of these conflicting forms are so close morphologically that one must rely almost wholly on biochemical reactions for the correct determination of the organism. On the other hand, fungi have been included in one of these groups which apparently have no generic similarity, making the literature abundant with misnomers.

In 1809 Link created the genus *Geotrichum* with the following characteristics (Link, Mag. Naturf. Ges. Fr. Berlin **3**: 17–18. 1809; Saccardo, Syll. Fung. **4**: 39. 1886): "Hyphae steriles repentes; fertiles breves, adscendentes septulatae. Conidia concatenata, breve cylindracea, utrinque truncata, hyalina."

Since the above description, numerous species have been added to the genus. The confusion existing in the literature has been briefly summarized by several authors, particularly Langeron and Talice ('32). From the genus *Mycoderma,* however, *Geotrichum* is to be differentiated particularly, since

these two groups have often been interchanged and even reduced to synonymy by Ciferri and Redaelli ('29).

Mycoderma has been considered to be similar in morphology to *Geotrichum.* However, there is greater gelification of the walls of *Mycoderma,* particularly at the cross-walls, the cells tending to become rounded or ellipsoidal as compared with the cylindrical cells of *Geotrichum,* where the ends remain abrupt or become somewhat rounded. This of course may vary in both genera, making morphological differentiation so difficult that biochemical and physiological reactions must be resorted to. In this respect, it is found that the Geotricha may produce a thick pellicle on liquid media; liquefy gelatine and serum, but do not ferment sugars, usually producing acidity and no gas. In the case of the Mycodermata, it is generally found that the colony surface on media is more folded, with no gelatine or serum liquefaction or fermentation of sugars.

As the majority of the members of the genus *Geotrichum,* as well as of *Mycoderma,* have been isolated from the soil, there are many saprophytes which are not as yet found to be pathogenic on man. In many cases, there have been determinations of fungi based on systems of classification which have either reduced several genera to synonymy or have adopted terms which in many cases do not apply to this group. A few of these may be briefly considered.

Sartory in 1907 placed these fungi in the genus *Oospora,* on the same footing as the Actinomycetes, and confirmed this in 1923 (Sartory et Bailly, '23). Castellani ('19) considered the fungus as *Oidium* Link, 1809, *emendavit* Pinoy, and defined it as "Oosporaceae with hyphae terminating in chains of spores. Hyphae long and branched. Sporophores simple, septate, often without disjunction apparatus. Do not produce gas in carbohydrates." He included four species as pathogens: *Oidium lactis* Link, 1809; *O. rotundatum* Castellani, 1911; *O. asteroides* Castellani, 1914; *O. matalense* Castellani, 1915. Since that time, numerous changes have occurred, and that author has published several new species and varieties of *Geotrichum,* whereas the four above fungi have been placed in *Geotrichum* by other writers. Some of these new organisms

may belong to the last-named group, while certain others are definitely not correct taxonomically.

Berkhout ('23) took up the name *Oospora* for these pathogenic fungi and included in it the genus *Oidium*. Ciferri and Redaelli refer the species to the Torulopsidaceae of the Mucedineae amerosporeae, on the same standing as the Oosporaceae and perhaps in synonymy with the latter. The Torulopsidaceae they further divide into the Torulopsideae, which replaces the former sub-family Cryptococcaceae, and the Mycotoruleae, including thus *Geotrichum,* which they use in preference to *Oospora* and *Mycoderma.* Vuillemin ('31), on the other hand, prefers to keep the group in *Mycoderma,* while Langeron and Talice ('32) classify it with the Mycotorulaceae of Ciferri and Redaelli, placing the genus in their *Geotrichoides,* a sub-group of the family which forms membranaceous colonies and comprises the single genus *Geotrichum.* They characterize the genus as having a true mycelium that breaks up into arthrospores with occasional blastospores, and forming a thick veil on liquid media.

A review of the literature revealed further that very few well-defined species of *Geotrichum* have been reported pathogenic for man. The several species known in literature: *G. pulmoneum* (Bennett) Basgal ('31), *G. asteroides* (Castellani) Basgal ('31), *G. louisianoideum* and *G. multifermentans* Castellani ('33), and several other fungi appearing in such genera as *Mycotorula, Torula, Oidium, Mycoderma, Oospora* and *Monilia,* which from their morphological and biochemical properties should be in *Geotrichum,* have definite characteristics which distinguish them, and possibly not all are correctly named. In addition, two organisms have been published recently with a generic change, namely *G. immite* (Rixford and Gilchrist) Agostini ('32) and *G. dermatitidis* (Gilchrist and Stokes) Castellani ('33). These fungi are of particular interest, since neither one presents characteristics identical with *Geotrichum. Geotrichum immite,* the cause of coccidioidal granuloma, furthermore, has been shown to have an ascus in its life cycle which immediately eliminates it from the above group and places it in the Ascomycetes where the author

('32) created the family Coccidioideaceae, with *Coccidioides immitis* as the type genus and species. The second fungus, *G. dermatitidis,* the cause of the American type of blastomycosis, has absolutely no association with the genus. This was also found to have an ascus with 8 spores in its life cycle and was consequently transferred by the author ('33, '33a) to the genus *Endomyces.*

After carefully studying the description of the above species, particularly of *Geotrichum* and the possibly related species in *Mycoderma, Oidium* and *Oospora,* the author concludes that the organism described in this paper should be a new species, *Geotrichum versiforme.*

Geotrichum versiforme Moore, n. sp.

Differs from other species of the genus *Geotrichum* by having many forms on various media. Macroscopically it varies from a velvety plush-like, to a moist, flat, asteroid or vermiculate condition. Color varies from a dull grayish-white to a dull creamy-buff with a "duvet" appearance, as on Sabouraud's and glycerine agar. Microscopically the cells vary in size, proportion, and development; hyphae 3–8 μ in diameter, with young cells approximately 6–40 μ long; arthrospores 4–9 x 6–18 μ; spherical chlamydospores 4–18 μ in diameter, elongated ones 6–8 x 20–30 μ; small spherical cells 4–6 μ in diameter, possibly blastospores; conidium-like cells, spherical 4–6 μ in diameter, pyriform 3–4 x 4–6 μ. No fermentation on any sugar. Acid and no gas after 2 days on 1-xylose, galactose, d-mannose, levulose, and maltose. No acid or gas on l-arabinose, rhamnose, dextrose, lactose, sucrose, raffinose, and inulin. Plain gelatine liquefies slowly at the surface after 12 days, and beef extract gelatine after 14 days.

Geotrichum versiforme Moore, spec. nov.

Cellulae plures figuras in mediis diversis habent. Coloniae inter se a panno-villosis holosericisque vel humidis, planis, stellatis vel vermiculatis diversae sunt. Superficies impolita, color albidus vel subalutaceus. Cellulae forma et magnitudine variantes. Hyphae diametro 3–8 μ; cellulae iuniores 6–40 μ longae; arthrosporae 4–9 x 6–18 μ; chlamydosporae sphaericae,

diametro 4–18 μ, chlamydosporae elongatae 6–8 x 20–30 μ; cellulae parvae sphericae 4–6 μ diametro quae blastosporae fieri possunt; cellulae conidio similes, globosae, diametro 4–6 μ, piriformes 3–4 x 4–6 μ. Fermentatio nulla. Acidus per biduum in "l-xylose, galactose, d-mannose, levulose, et maltose." Acidus nullus in "l-arabinose, rhamnose, dextrose, lactose, sucrose, raffinose, et inulin." Gelatina communis per duodecim diebus in summa cuto fluidificans, gelatina decocta bubula per quattordecim diebus fluidificans.

Summary and Conclusions

1. A case of bronchiectasis and pulmonary infiltration is reported, from which was isolated an organism identified as a new *Geotrichum*.

2. The fungus, grown in hanging-drops, may develop from an arthrospore to form hyphae, either single or branched, which at maturity form cross-walls and thick walls, breaking up into arthrospores by disarticulation.

3. When grown on a variety of standard media, differing in pH, protein and carbohydrate content, the organism showed different forms on different substrates, developing the largest cells on malt extract agar. The colonies varied from a furry or plush-like growth to a "duvet," flat, moist or vermiculate growth. The color varied from a grayish-white to a creamy-buff.

4. There is no fermentation of any sugar. Acidity is produced with l-xylose, galactose, d-mannose, levulose, and maltose, while an alkaline reaction takes place with l-arabinose, rhamnose, dextrose, lactose, sucrose, raffinose, and inulin. This latter observation may be explained by the breakdown of the amino acids of the medium to alkaline bases, as arginine, histidine and lysine, and also ammonia. Plain gelatine is liquefied after 12 days and beef extract gelatine after 14 days.

5. Because of the several forms on the different media, color production, carbohydrate reactions, and gelatine liquefaction, the organism is described as a new species, *Geotrichum versiforme* Moore.

Acknowledgments

The author is indebted to Dr. Carroll W. Dodge, Professor of Botany in the Henry Shaw School of Botany of Washington University, for the use of his unpublished manuscript and data; to Dr. George T. Moore, Director of the Missouri Botanical Garden, for the facilities of the mycological laboratory and the library; and Dr. Ralph S. Muckenfuss, Assistant Professor of Medicine, Washington University School of Medicine, for the culture of the organism here described.

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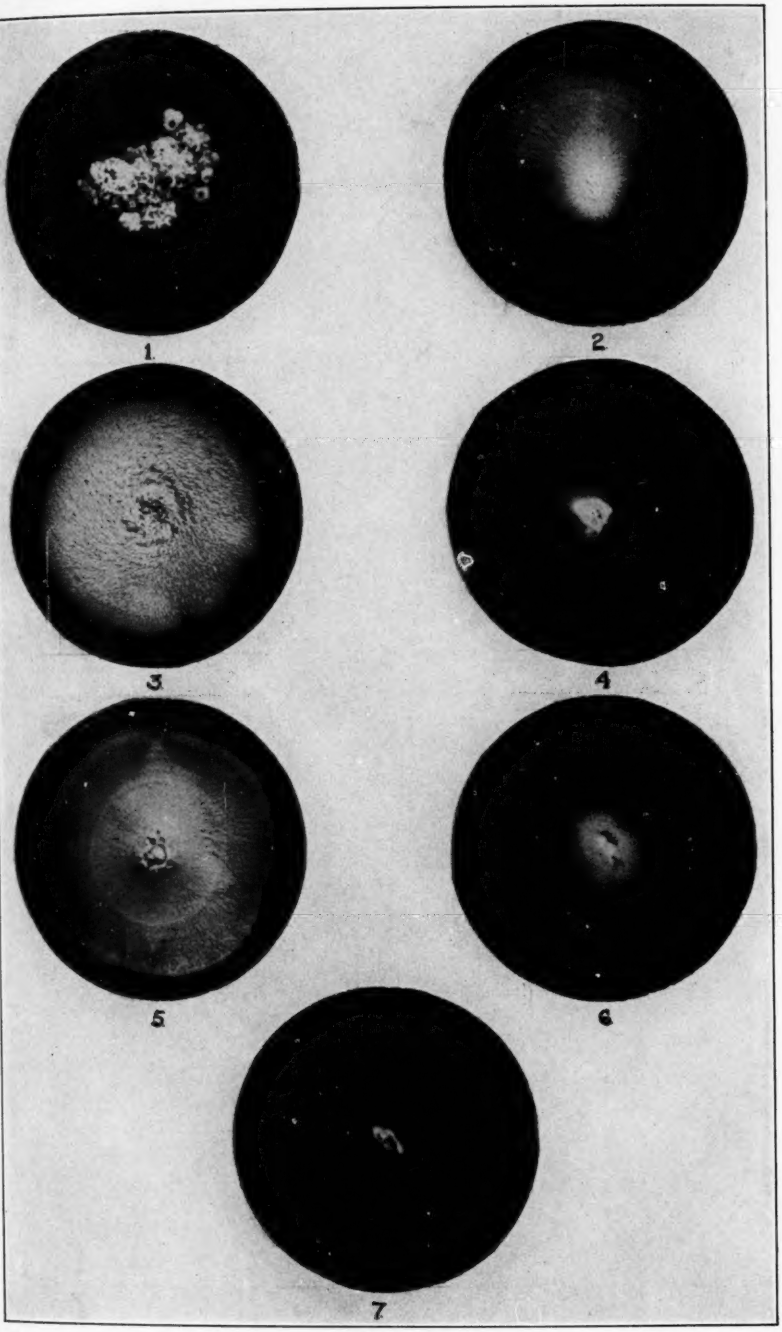
Explanation of Plate

PLATE 15

Geotrichum versiforme

Photographs of colonies, 8 days old, on various media.

Fig. 1. Malt extract agar, pH 5.2. × 1.
Fig. 2. Sabouraud's agar, pH 5.6. × ⅝.
Fig. 3. Potato-dextrose agar, pH 5.9. × ⅝.
Fig. 4. Lactose agar, pH 6.8. × 1.
Fig. 5. Glycerine agar, pH 7.0. × ⅞.
Fig. 6. Nutrient agar, pH 7.2. × ⅞.
Fig. 7. Endo's agar, pH 7.5. × ⅝.



MOORE—GEOTRICHUM VERSIFORME

EXPLANATION OF PLATE

PLATE 16

Geotrichum versiforme

All figures drawn as correctly as possible at a magnification of $\times 960$ and reduced to $\times 500$, with the aid of a camera lucida.

Fig. 1. Simple arthrospore before germination, on Raulin's agar.

Fig. 2. Germinating arthrospore on Richards' agar.

Figs. 3–5, 7. Germinating arthrospores on Sabouraud's glucose agar.

Fig. 6. Germinating arthrospore on nutrient agar.

Fig. 8. Young filament on glycerine agar.

Fig. 9. Hypnospore-like cell on Sabouraud's glucose agar.

Fig. 10. Young filament showing vacuoles of future arthrospores, on Czapek's agar.

Fig. 11. Mycelium with terminal arthrospores and chlamydospores on malt extract agar.

Fig. 12. Mycelium on potato-dextrose agar.

Fig. 13. Large round chlamydospores on malt extract agar.

Fig. 14. Mycelium showing formation of arthrospores and a conidium-like cell on Czapek's agar.

Fig. 15. Type of cross-wall present on mycelium grown in Sabouraud's broth.

Fig. 16. Mycelium showing pyriform, conidium-like cell on Sabouraud's glucose agar.

Fig. 17. Young mycelium showing vacuoles and arthrospore formation in lactose broth.

Fig. 18. Chains of arthrospores on Raulin's agar.

Fig. 19. Mycelium showing large round chlamydospores, arthrospores, and sclerotic cells on malt extract agar.

Fig. 20. Mycelium showing septal formation and sclerotic cells on malt extract agar.

Fig. 21. Group of rectangular arthrospores on corn-meal agar.

Fig. 22. Mycelium showing arthrospores, rounded and rectangular, on Richards' agar.

Fig. 23. Barrel-shaped arthrospores and an intercalary chlamydospore on malt extract agar.

Fig. 24. Chain of ovoid to round arthrospores on Richards' agar.

Fig. 25. Arthrospore formation on nutrient agar.

Fig. 26. Cell showing cylindrical appearance, on Raulin's agar.

Fig. 27. Arthrospores formed terminally on a filament, on Sabouraud's maltose agar.

Fig. 28. Chain of round cells on Richards' agar.

Fig. 29. Pyriform terminal cell on Raulin's agar.

Fig. 30. Branching filament with arthrospores on Sabouraud's maltose agar.

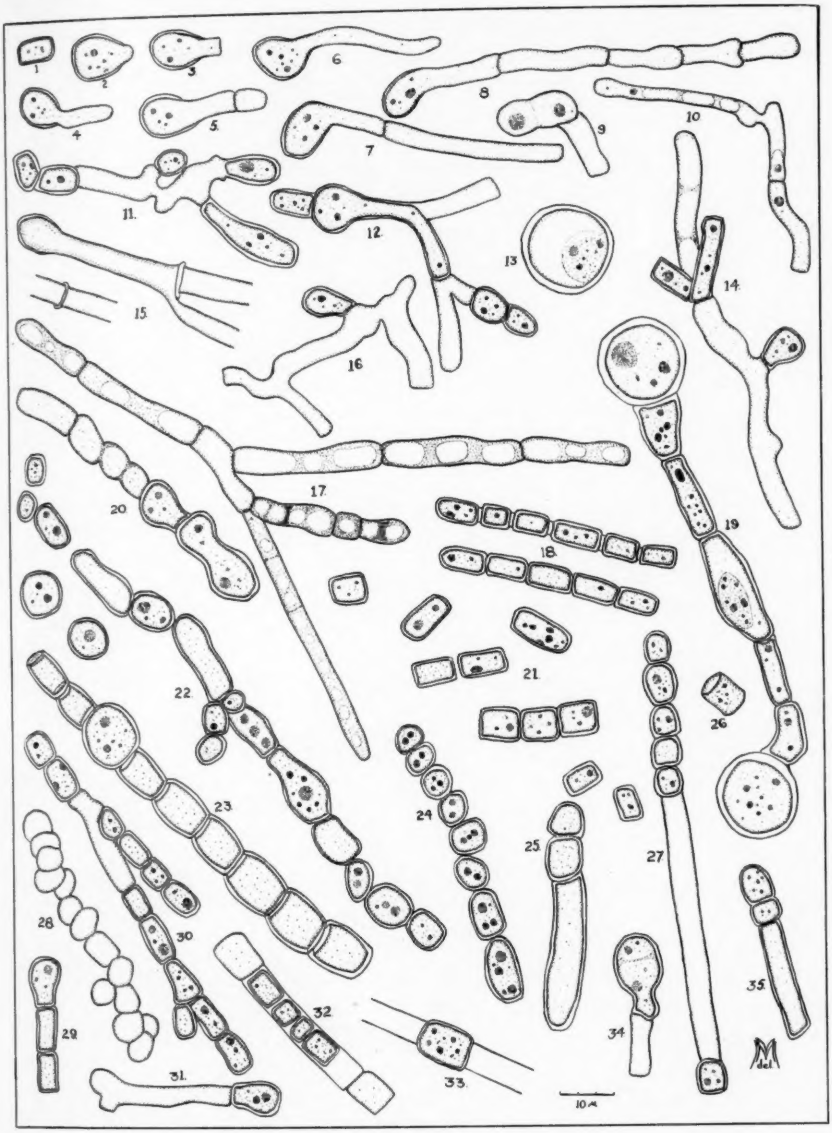
Fig. 31. Pyriform chlamydospore on Czapek's agar.

Fig. 32. Arthrospores formed within a filament on corn-meal agar.

Fig. 33. Chlamydospore comparable to an akinete as found in algae, on corn-meal agar.

Fig. 34. Terminal chlamydospore on nutrient agar.

Fig. 35. Terminal arthrospores on malt extract agar.



MOORE—GEOTRICHUM VERSIFORME

THE EFFECTS OF INCREASING THE IODINE CONTENT OF THE TOMATO PLANT ON RESPIRATION AND ENZYMATIC ACTIVITY[1]

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I. Introduction

Ever since its discovery in kelp by Courtois in 1811, iodine has been the subject of much biological research, and a truly

[1] An investigation carried out in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University and submitted as a thesis in partial fulfillment of the requirements for the degree of doctor of philosophy in the Henry Shaw School of Botany of Washington University.

enormous literature has developed. The greater number of studies deals with the relation of iodine to animal metabolism, especially to the activity of the thyroid gland. Even before its discovery in the thyroid glands by Baumann ('95), its connection with goitre was suspected, and Fourcault ('51 a, b), Thenard ('51), Chatin ('51–'53), Grange ('52), Casaseca ('53) and others had published their results on the relation of the iodine content of food, water, and air to the prevalence of human goitre and cretinism.

According to Muller (Lindley's 'The Vegetable Kingdom,' p. 353) iodine had been found in a cress of unknown origin. This fact was investigated and verified by Chatin ('50 a, b, '66), who may be considered the first to determine positively iodine in the higher plants. Since that time many questions have been raised concerning possible functions of iodine in the plant, but studies attacking these problems have been of little significance. The pertinent literature is so extensive that a satisfactory review can not be attempted here.

The unsatisfactory results of iodine research in plant physiology are perhaps due primarily to the lack of a successful method of growing plants free from iodine, and the absolute necessity of this element to growth is therefore almost impossible to demonstrate. Iodine is omnipresent in the air, according to Chatin ('51–'66), Gautier ('99, '20), and Wagner ('29), and also in water and soil, at least in minute traces. Even if the external experimental habitat were freed from iodine, the seed itself might pass on from generation to generation a biological sufficiency of this element.

The present paper reports the results of a study on tomato plants grown in water cultures to which various amounts of iodine as potassium iodide were added. The effects of the increasing amounts of iodine on growth, toxic symptoms, respiration, and enzymatic activity were observed.

Many workers have found that plants absorb iodine more or less proportionally to the amount present in the soil or culture solution. Stoklasa ('24) grew sugar beets in pots containing 12 kilograms of soil to which .02 gram iodine was added as potassium iodide, and found that in the air-dry tissue the con-

trols contained .32 milligram iodine per kilogram of leaves, and .15 milligram per kilogram of roots, while the plants grown in the iodized pots contained .90 milligram iodine per kilogram of leaves and .60 milligram per kilogram of roots. Other researchers also have found an increase in iodine content on addition of this substance to the substrate, as Stoklasa ('26, '29, '30), Scharrer and Schwaibold ('27), Scharrer and Strobel ('27), Orr, Kelly, and Stuart ('28), Hiltner ('28). The field studies of Wrangell ('27) did not show any increase in iodine with iodine manuring, but as was later pointed out by Orr, Kelly, and Stuart ('28) this may have been due to some abnormal soil condition which made the iodine unavailable. It should be noted also that Klein ('27) in his critical review concluded that the iodine content of plants was held between narrow limits and that it was not possible to vary this significantly. In spite of these dissenting voices, it seems reasonable to conclude that the amount of iodine in plant tissues is dependent to a greater or less degree on the amount available in the substrate. Owing to the fact that all of the various enzymes discussed below were studied from a single lot of plants in order that the conditions of growth would be identical, sufficient tissue was not available for iodine analysis. The maximum possible number of plants was grown in the time permitted, and the tissue proportioned to the different phases of the work. Since the literature gives sufficient evidence concerning the absorption of iodine, all of the tissue available was reserved for the enzyme studies. The results reported below indicate definite physiological effects varying with the different concentrations of potassium iodide in the nutrient solution. This is in itself a proof that increasing amounts of iodide were absorbed. Kostytschew ('26) summarizes the situation as follows: "Es ist festgestellt, dass fermentative Oxydations- und Gärungsvorgänge durch Alkaloide und andere Reizstoffe ausserhalb der Zelle nicht gesteigert werden können. Nun ist auch eine Fermentbildung ausserhalb des lebenden Plasmas bisher nicht bekannt."

II. General Methods

"Bonny Best" tomato seed were planted February 27, 1932, in flats of greenhouse soil, and the seedlings were transplanted to the water culture jars on March 25. It is, of course, possible that during this month of contact with soil, sufficient iodine was absorbed to mask the effect of the later addition of small amounts to the water cultures. However, the purpose of the work was not to study the necessity of iodine in itself, nor to determine its optimum concentration for growth, but to observe the physiological effects of increasing the iodine content of the plant. Hence the possibility of the absorption of some iodine during this stage was disregarded, since all seedlings were germinated under identical conditions, in the same greenhouse flat.

The nutrient medium used was Shive's ('17) three-salt solution, which he found best for buckwheat tops. Its composition was as follows:

KH_2PO_4	.0144 M
$Ca(NO_3)_2$	.0052 M
$MgSO_4$	.0200 M

One part per million of manganese as manganous sulphate, boron as sodium borate, and iron as ferrous sulphate were used in all cultures. Potassium iodide was added to give 1, 5, 10, and 20 parts per million of iodine. A control series contained no potassium iodide.

The cultures were grown in one-quart glazed earthenware jars. These were allowed to stand filled with 2N sodium hydroxide for two weeks and with 2N sulphuric acid for two weeks, so that all easily soluble substances would be dissolved from the surface. Sheets of cork six inches square and one-half inch thick covered the tops of the jars so that no light could reach the roots. Holes one-half inch in diameter were cut in the cork to accommodate the plants. Six plants were placed in each jar. During the first week the solutions were renewed twice, during the second week three times, and daily from then on.

III. Experimental Results

A. Growth

The earliest worker dealing with the effect of iodine on the growth of plants was Suzuki ('02b). He grew *Pisum* in pots containing 2300 grams of air-dry soil plus .001 grain potassium iodide added six times during the growth period. The iodized soil was reported as causing the weight of fresh fruits to increase from 60.5 to 72.4 grams, the air-dry seed to increase from 23.2 to 26.3 grams, and the air-dry straw to increase from 10.7 to 15.5 grams. Since only one pot each was used for the control and experimental plants, and each pot contained only five plants, it is not possible to draw any positive conclusions from the experiment. The following year Susuki and Aso ('03) reported a stimulation of radish and oat seedlings in pot culture by the addition of potassium iodide. Here again the number of plants was too small to lend significance to the results.

Mazé ('15), noticing that corn grew well in sterile spring water but imperfectly or not at all in distilled water, set out to find the essential elements of the nutrition of this plant. The elements ordinarily present in nutrient solution did not give good growth. The addition of .004 grams of potassium iodide per liter showed a beneficial effect.

Budington ('19) observed the growth of onion root tips in 20 cc. of Pfeffer's nutrient solution to which .25 to 1 grain of desiccated thyroid gland was added. Only harmful effects on growth were noted, although when the same amounts of iodine were added as potassium iodide the growth was normal, from which it appears that the thyroid was harmful for some reason other than its iodine content.

Stoklasa ('24) found in pot studies that the addition of .02 gram of iodine as potassium iodide to 12 kilograms of soil produced a better development of sugar beets, especially of the leaves. In field studies 1.72 kilograms of iodine as potassium iodide per hectare gave a better growth of sugar beets and caused more and better seeds to develop during the second year. Stoklasa ('26) also found that .009–.015 gram of iodine as

potassium iodide per 3200 cc. of nutrient solution increased the growth of *Hemerocallis fulva* almost 100 per cent. Potassium iodide at the rate of .021 gram of iodine per 12 kilograms of earth increased the growth of the leaves and roots of the sugar beets, confirming some earlier observations. Dafert and Brichta ('26), in comparing the value of synthetic nitrate with Chile saltpeter, found that iodine in an amount equal to that in the natural product had no effect on barley, mustard, and turnips. Brenchley ('24) found little or no beneficial effect of an iodine-potassium-iodide mixture on barley or mustard, but suggested that other plants might react differently. Wrangell ('27), using .3–1.5 kilograms of iodine as potassium iodine per hectare, grew a variety of agricultural plants, with no apparent effect on growth. Haas and Reed ('27) found that young orange trees did not thrive after 18–24 months unless the nutrient solution contained .2 part per million of I, Al, Ti, Br, Sr, Si, Mn, B, NH_4. It does not follow that each of these elements is necessary.

Scharrer and Schwartz ('27) found that small amounts of various inorganic iodine compounds slightly stimulated the multiplication of yeast, but did not increase the maximum yield. This work was followed by that of Greaves, Zobell, and Greaves ('28), who obtained poor growth on iodine-free nutrient solution and vigorous growth with 10 parts per million of iodine as potassium iodide. These authors held iodine to be an essential nutrient for yeast.

Engles ('28), through field studies on sugar beets and turnips, concluded that there was no stimulative effect from iodine added as potassium iodide. Stoklasa ('29) again reported a large increase in the growth of sugar beets from potassium iodide manuring. According to Haas ('30), iodine could be omitted from nutrient solutions for citrus plants with no harmful effect. Cotton ('30) found no stimulation to buckwheat growth in nutrient solutions containing concentrations of potassium iodide of 1.27 to 12.7 parts per million of iodine. Scharrer and Schropp ('31) observed a slight stimulation at

low concentrations of iodine compounds on wheat in pot studies.

From this incomplete review of the literature it is apparent that no agreement has yet been reached concerning the effect of iodine on the growth of plants.

In the present work, only depressing effects on growth were observed. The average of 25 plants after a growth period of 2 months is represented in fig. 1, and the numerical data appear in tables I and II. From the curves it is seen that both the

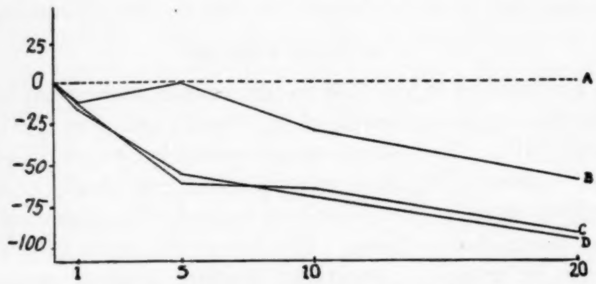


Fig. 1. Growth data: A, succulence (% of loss in drying); B, dry weight of roots; C, dry weight of tops; D, fresh weight of tops.

fresh and dry weight of the tops were about equally depressed. The dry weight of the roots was less depressed, and then only at the higher concentration. The degree of succulence as shown by the loss of weight on drying is apparently unaffected.

TABLE I

GROWTH DATA IN ABSOLUTE AMOUNTS. AVERAGE BASED ON 25 PLANTS. VALUES TAKEN ON APRIL 27, 1932, AFTER A GROWTH PERIOD OF 2 MONTHS

Concentration of Iodine	Fresh wt. of tops (gms.)	Dry wt. of tops (gms.)	Dry wt. of roots (gms.)	Loss of wt. of tops in drying (gms.)	% of loss in tops by drying
Control	9.4	.84	.28	8.56	91
1 p.p.m.	8.7	.78	.26	7.92	91
5 p.p.m.	6.8	.59	.28	6.21	91
10 p.p.m.	6.2	.57	.24	5.63	91
20 p.p.m.	5.0	.46	.20	4.54	91

TABLE II

SAME AS TABLE I, THE FIGURES INDICATING THE RELATIVE VALUES, UPON WHICH THE GRAPHS OF FIGURE 1 ARE BASED. THE CONTROL IN EACH CASE IS TAKEN AS 100

Concentration of Iodine	Fresh wt. of tops	Dry wt. of tops	Dry wt. of roots	% of loss in tops by drying
Control	100	100	100	100
1 p.p.m.	92	93	93	100
5 p.p.m.	72	70	100	100
10 p.p.m.	66	68	85	100
20 p.p.m.	53	55	71	100

B. TOXIC SYMPTOMS

The evidence of injury due to toxic concentrations of iodine compounds has been described by Suzuki and Aso ('03) and by Mazé ('19). These authors observed brown spots on the foliage. Cotton ('30), in a paper dealing specifically with the toxic effects of iodine and nickel on buckwheat in water culture, verified these observations. She found the spots to be characteristic in aspect. Generally slightly sunken areas appeared first, often near the margins of the leaves. These became pale brown spots encircled by a darker brown, and the areas enlarged and coalesced. The petioles then turned brown at the base and the leaf dropped.

In the present work many hundreds of tomato plants were grown in concentrations of potassium iodide that were obviously toxic to the plant, but no brown spotting of the foliage was observed. At a concentration of 1 part per million of iodine as potassium iodide, there was no visible effect on the health of the plant. At 5 parts per million, only rarely did individual plants appear to be unhealthy. Concentrations of 10 and 20 parts per million usually gave plants unhealthy in appearance. Toxicity was first shown by a decrease in the intensity of the green color. This was followed by a definite chlorosis of the leaves, particularly of the lower. It is true that this chlorotic condition developed first in the tissue between the larger veins and later spread over the entire leaf, but strictly speaking these areas could not be defined as spots.

This difference is probably due to the specific reaction of tomato plants, although it is noted that Mazé worked with corn and Cotton worked with buckwheat, and both obtained a characteristic spotting of the leaves. The leaves of our plants were quickly dropped when the chlorotic condition was reached. No specific injury to the growing tips was noted.

C. ACIDITY OF THE PRESS JUICE

Stoklasa ('26) grew *Senecio vulgaris* in pot culture, with the addition of 0.05 gram of iodine as potassium iodide to each 9.5 kilograms of soil. After 40 days the pH of the press juice was determined. The iodine-manured plants showed a conspicuous increase in pH over the controls. The same result was obtained with *Epilobium hirsutum* and with *Beta vulgaris*. This change in acidity he attributed to the breaking down of organic acids due to the iodine in the plant increasing the enzymatic decomposition of these substances.

Since Stoklasa attributed considerable importance to this decrease in acidity because it would tend to stimulate general enzymatic activity in the tissue, a careful study was made of this feature. The press juice from 10 plants was determined by the quinhydrone electrode for each concentration of iodine in the nutrient solution. No significant difference was observed, and the variation in enzymatic activity of the tomato plants described below must be attributed to some other action than the effect of the iodine on the acidity of the press juice.

D. RESPIRATION

The effect of iodine and iodine compounds on animal respiration has been investigated by a number of authors. Macht and Hooker ('18) studied the action of iodide, bromide, and nitrate ions on the respiratory center of a small dog. Dog's blood was defibrinated and diluted with Locke's solution in which the usual sodium chloride was replaced by sodium iodide. This preparation was perfused into the carotid artery under such conditions that the profusate was practically isolated in the head region. It was found that the iodide ion, as well as the bromide ion, stimulated the respiratory rate, as shown by the deeper amplitude of the respiration curve. Stimulation of the

respiratory center is equivalent to an increase in intracellular oxidation only in case its activity parallels the demand by the cells for oxygen.

Cameron and Carmichael ('20) found that continued small doses of desiccated thyroid fed to white rats caused, among other things, a hypertrophy of various organs due to an increased metabolic rate. Sodium iodide fed in amounts equaling the iodine of the thyroid up to 100 times as much did not produce this effect, which indicates that it is not iodine itself but a particular iodine compound which is the effective agent.

Hara ('23) found that injections of elementary iodine into the rat in amounts corresponding to 2 grams in man did not affect the metabolic rate, as shown by the comparative oxygen absorption.

Wickwire, Seager, and Burge ('28) observed the effect of various iodine compounds on the rate of sugar utilization by goldfish. Desiccated thyroid, thyroxin, clacidine, potassium iodide, and sodium iodide, respectively, were added to 100-cc. portions of .1 per cent dextrose in such amounts that each portion contained .55 milligram of iodine. Two goldfish were put in each solution. Air was bubbled through, and after 30 hours the sugar remaining in the solution was determined by the Benedict method. Only the desiccated thyroid had any effect. This substance caused an increase of 50 per cent in the sugar utilization over that of the control. These results agree with those of Cameron and Carmichael ('20) that it is not iodine in itself but a definite compound of iodine that stimulates metabolism. Wilhelmj and Boothby ('30) further verified the stimulatory effects of thyroxin on the basal metabolic rate.

Baker, Bacon, Lundy and Klein ('30) found that a thyroid extract injected intravenously in dogs caused a depression of the respiration rate, followed by an increase. The same result was obtained with extracts of kidney, suprarenal gland, liver, pancreas, duodenum, thymus, prostate, and muscle, and therefore nothing can be inferred concerning the actual effect of

iodine. These authors held that the symptoms were not due to thyroxin nor to di-iodo-tyrosin, since none of these substances showed the same amount of iodine present in the tissue extracts.

The discussion of the relation between the function of the thyroid and metabolic activities, particularly in cases of human goitre, is too extensive to review here.

The influence of iodine and iodine compounds on the rate of plant respiration metabolism has been but little studied, in spite of the fact that the results obtained by the animal biologists suggests that this would be a fertile field for research.

Lieben and Lászlo ('25) investigated the effect of various ions on sugar metabolism of oxygenated yeast and found that the iodine ion, in common with several other ions, significantly increased the utilization of sugar although there appeared to be no relation between the quantity of these ions present and their effectiveness.

Stoklasa ('26) found that the roots of iodine-poor sugar beets produced 1463 milligrams CO_2 in a definite period, while iodine-rich roots eliminated 1522 milligrams during the same time. In anaerobic conditions and in a pure hydrogen atmosphere this increase did not occur. Comparable results were obtained with potato tubers. This author also held that iodine as potassium iodide in the nutrient medium of *Azotobacter chroococcum* stimulated respiration, and thereby increased the amount of nitrogen fixed during the process.

Greaves, Zobell and Greaves ('28) investigated the effects of iodine and iodine compounds on the rate of reproduction and carbon-dioxide output of yeast. Commercial yeast in the presence of 1 part per million of iodine as potassium iodide showed an increased growth, but the individual metabolic activity was not affected. Comparable results were obtained with sodium iodide, calcium iodide, and iodine. The metabolic activity was increased, however, by these substances in the presence of maltose or lactose.

Scharrer and Claus ('30) found that 9-hour cultures of beer yeast eliminated less CO_2 when .5 per cent iodine as sodium

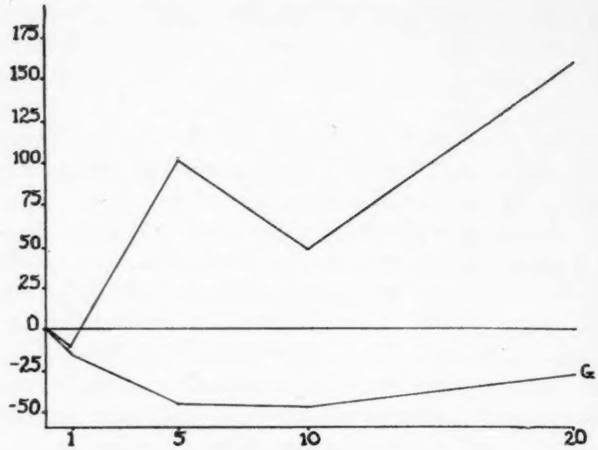


Fig. 2. Respiration Determination 1.

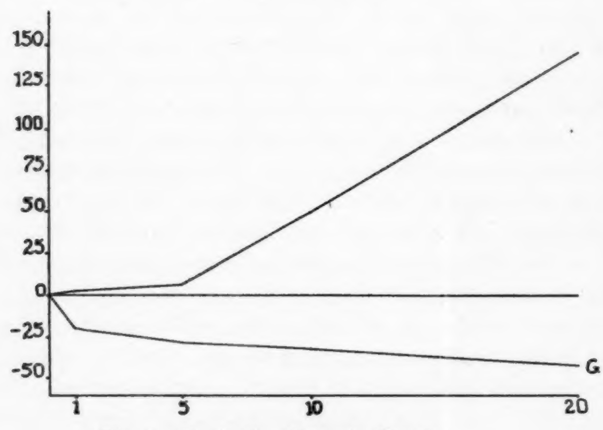


Fig. 3. Respiration Determination 2.

Explanation of graphs.—In all cases, the value of the control plant is calculated as 100, and the potassium-iodide-treated plants as per cents of the control. The numbers on the ordinate represent the per cent stimulation or depression, the graphical value of the control therefore being zero. The horizontal line divides the stimulation and depression values. The numbers on the abscissa represent the parts per million of iodine as potassium iodide in the nutrient solution. Curve "G" represents the growth of the plants used for the determinations indicated by the remaining curves of the same figure.

iodide was present in the culture medium, but with 2.02 per cent iodine there was a conspicuous increase.

As a part of the present investigation the carbon dioxide output from tomato plants growing in water culture was measured. In preliminary work it was found difficult to obtain thorough absorption of carbon dioxide by drawing air through liquid absorbents unless such a quantity of absorbents was used that accurate titration was impossible. Absorption bulbs so constructed that the air bubbles were broken several times gave complete absorption, but the absorbent was difficult to remove completely after each determination. Since liquid absorbents were either inaccurate or inconvenient, an apparatus involving a dry carbon-dioxide absorbent was designed. This has been previously reported by the author ('32), and is pictured in plate 17.

In each case, the nutrient solutions were renewed just before the determinations were started in order to eliminate the possibility of inaccuracy due to the activity of microorganisms. The culture jars, each containing from four to six plants, were placed in the bell-jar compartments of the respirometer at 10 o'clock in the evening, and the room was thoroughly darkened to eliminate photosynthetic activity during the early part of the succeeding morning. (For the details of the apparatus and of the procedure of determination, see Wynd, '32). At 8 or 9 o'clock the next morning, the Fleming-Martin absorption bulbs were disconnected and weighed on the analytical balance. The plants were then weighed and the carbon-dioxide elimination per gram of fresh weight of tops was calculated (tables III–IX). Since the relation between the extent of iodine injury and the increase in respiration is of some interest, detailed descriptions are given below.

RESPIRATION DETERMINATION 1

Control—Plants normal in color and appearance.

1 p.p.m.—Plants indistinguishable from the controls.

5 p.p.m.—Plants showing slight injury; less green than the preceding; the lower leaves dropped.

10 p.p.m.—Indistinguishable from plants in the 5 p.p.m. solution.

20 p.p.m.—Indistinguishable from plants in the 5 p.p.m. solution.

Examination of table III and fig. 2 shows that the increase in respiration is not closely correlated with the extent of injury. Attention may be called to the fact that the plants growing in 20 p.p.m. of iodine showed even less injury as shown by the growth curves, than those in the two preceding concentrations, although their respiration was conspicuously higher.

TABLE III

RESPIRATION DETERMINATION 1, DATA TAKEN APRIL 18, 1932

Iodine concentration	Number of plants	Average fresh wt. of tops (gms.)	Relative fresh wt. of tops (control as 100)	CO_2 output in 11 hrs. per gm. fresh wt. (gms.)	Relative CO_2 output per gm. fresh wt. (control as 100)
Control	6	8.90	100	.00321	100
1 p.p.m.	6	7.54	85	.00289	99
5 p.p.m.	5	5.10	57	.00637	199
10 p.p.m.	6	4.87	55	.00476	148
20 p.p.m.	6	6.64	74	.00842	262

RESPIRATION DETERMINATION 2

Control—Plants entirely normal in color and appearance.
1 p.p.m.—No visible toxic effect; plants entirely similar to the control.
5 p.p.m.—No visible toxic effect; plants entirely similar to the control.
10 p.p.m.—No visible toxic effect; plants entirely similar to the control.
20 p.p.m.—Plants less green, but decrease in color so slight as to be almost indetectable by the eye; a few of the lower leaves dropped.

Examination of table IV and fig. 3 shows that very little toxic effect was shown in this series. A small decrease in fresh weight occurred at 1 p.p.m., but no additional decrease oc-

TABLE IV

RESPIRATION DETERMINATION 2, DATA TAKEN APRIL 19, 1932

Iodine concentration	Number of plants	Average fresh wt. of tops (gms.)	Relative fresh wt. of tops (control as 100)	CO_2 output in 10 hrs. per gm. fresh wt. (gms.)	Relative CO_2 output per gm. fresh wt. (control as 100)
Control	6	10.3	100	.00366	100
1 p.p.m.	6	8.3	81	.00374	102
5 p.p.m.	5	7.4	72	.00388	106
10 p.p.m.	6	7.0	68	.00543	149
20 p.p.m.	6	6.0	58	.00904	247

curred at the higher concentrations. Again there appears to be no striking connection between respiratory activity and degree of injury.

RESPIRATION DETERMINATION 3

Control—Entirely normal plants.
1 p.p.m.—Entirely normal, indistinguishable from the controls.
5 p.p.m.—Entirely normal, indistinguishable from the controls.
10 p.p.m.—Lower leaves slightly yellowish.
20 p.p.m.—Entire foliage very slightly chlorotic.

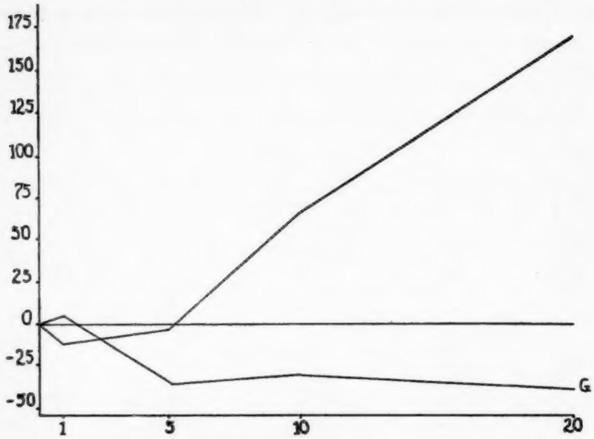


Fig. 4. Respiration Determination 3.

In this series, the 1 p.p.m. plants, while indistinguishable from the controls in appearance, weighed a little more. The data are given in table v and fig. 4. There is obviously no injury

TABLE V

RESPIRATION DETERMINATION 3, DATA TAKEN APRIL 20, 1932

Iodine concentration	Number of plants	Average fresh wt. of tops (gms.)	Relative fresh wt. of tops (control as 100)	CO_2 output in 10 hrs. per gm. fresh wt. (gms.)	Relative CO_2 output per gm. fresh wt. (control as 100)
Control	6	9.5	100	.00569	100
1 p.p.m.	6	9.9	104	.00455	89
5 p.p.m.	6	6.4	67	.00552	97
10 p.p.m.	6	6.7	71	.00924	164
20 p.p.m.	5	6.1	63	.01548	270

here, but again the respiration at this point is decreased. At higher concentrations, the increase of respiration was not proportional to the degree of visible injury nor to the decrease in weight. This is clearly shown in fig. 4.

RESPIRATION DETERMINATION 4

Control—Plants entirely normal.
1 p.p.m.—Plants entirely normal, similar to the controls.
5 p.p.m.—Lower leaves slightly yellowish.
10 p.p.m.—Plants appearing identical to those in 5 p.p.m.
20 p.p.m.—Whole plant slightly less green than controls; lower leaves dropped.

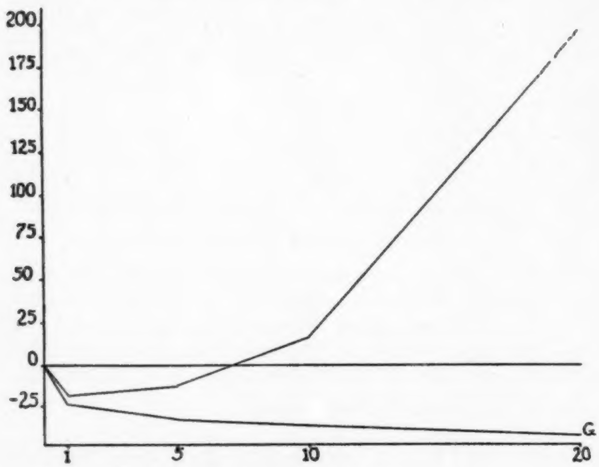


Fig. 5. Respiration Determination 4.

TABLE VI

RESPIRATION DETERMINATION 4, DATA TAKEN APRIL 21, 1932

Iodine concentration	Number of plants	Average fresh wt. of tops (gms.)	Relative fresh wt. of tops (control as 100)	CO_2 output in 10 hrs. per gm. fresh wt. (gms.)	Relative CO_2 output per gm. fresh wt. (control as 100)
Control	6	9.2	100	.00349	100
1 p.p.m.	6	7.4	79	.00278	80
5 p.p.m.	6	6.4	68	.00300	86
10 p.p.m.	6	6.0	64	.00403	116
20 p.p.m.	5	3.7	39	.01046	299

The plants of this series showed a more definite visible injury at the three higher concentrations than did any of the preceding series, although the respiration curve is entirely similar to that of determination 3, the plants of which showed almost no injury. The data are shown in table VI and fig. 5.

RESPIRATION DETERMINATION 5

Control—Plants normal.
1 p.p.m.—Slightly less green than controls.
5 p.p.m.—Less green than the preceding; some lower leaves dropped.
10 p.p.m.—Less green than the preceding; more of the lower leaves dropped.
20 p.p.m.—Definitely chlorotic, and quite stunted; all lower leaves dropped.

The visible toxic symptoms do not correspond necessarily to the decrease in weight, as is shown by table VII and fig. 6.

TABLE VII

RESPIRATION DETERMINATION 5, DATA TAKEN APRIL 22, 1932

Iodine concentration	Number of plants	Average fresh wt. of tops (gms.)	Relative fresh wt. of tops (control as 100)	CO_2 output in 10 hrs. per gm. fresh wt. (gms.)	Relative CO_2 output per gm. fresh wt. (control as 100)
Control	6	8.2	100	.00472	100
1 p.p.m.	6	6.1	74	.00450	95
5 p.p.m.	5	6.6	80	.00528	112
10 p.p.m.	5	8.7	106	.00580	123
20 p.p.m.	4	3.3	40	.01588	332

RESPIRATION DETERMINATION 6

Control—Entirely normal.
1 p.p.m.—Entirely healthy and visibly larger than the controls.
5 p.p.m.—Lower leaves yellowish.
10 p.p.m.—Entire foliage yellowish; lower leaves dropped.
20 p.p.m.—More yellowish than the preceding, and more of the lower leaves gone.

Here, as in determination 3, the 1 p.p.m. plants were better than the controls, but the respiration at this point was lower as shown in table VIII and fig. 7.

The average of all determinations is represented in table IX and fig. 8. The absolute values given in the tables for the different sets of determinations are not strictly comparable. The conditions of the determinations varied as to temperature, length of the experimental period, and the relative degree of

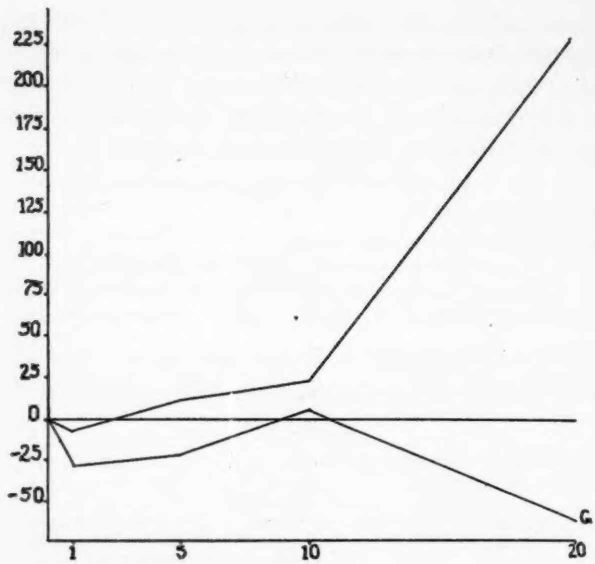


Fig. 6. Respiration Determination 5.

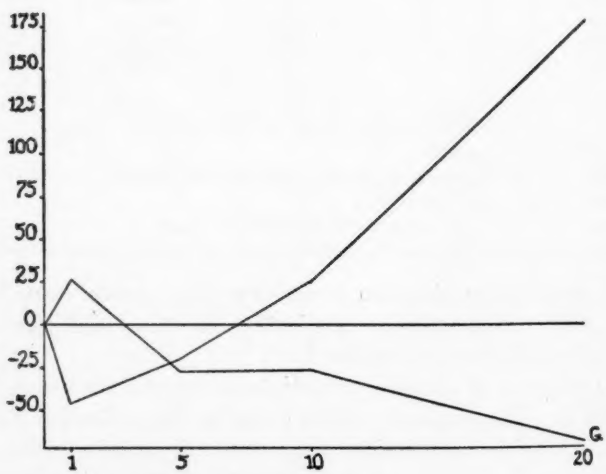


Fig. 7. Respiration Determination 6.

TABLE VIII

RESPIRATION DETERMINATION 6, DATA TAKEN APRIL 25, 1932

Iodine concentration	Number of plants	Average fresh wt. of tops (gms.)	Relative fresh wt. of tops (control as 100)	CO_2 output in 11 hrs. per gm. fresh wt. (gms.)	Relative CO_2 output per gm. fresh wt. (control as 100)
Control	6	9.1	100	.00464	100
1 p.p.m.	6	11.5	126	.00246	53
5 p.p.m.	5	6.9	75	.00371	80
10 p.p.m.	6	7.0	76	.00596	128
20 p.p.m.	5	3.2	35	.01320	284

iodine injury. However, the conditions within any one set of determinations were identical since they were carried out simultaneously. It is for this reason that the graphs are drawn on the basis of the relative values within a given set. It is probably not justifiable to give absolute values for the averages of determinations under different conditions. The average of the relative values does give, however, a general picture of the reactions under the various sets of conditions.

TABLE IX

AVERAGE FOR ALL RESPIRATION DETERMINATIONS

Iodine concentration	Number of plants averaged	Average fresh wt. of tops (gms.)	Relative fresh wt. of tops (control as 100)	Average CO_2 output in 10 hrs. per gm. fresh wt. (gms.)	Average relative CO_2 output per gm. fresh wt. (control as 100)
Control	36	9.2	100	.00424	100
1 p.p.m.	36	8.4	91	.00349	85
5 p.p.m.	32	6.7	73	.00463	113
10 p.p.m.	35	6.7	73	.00587	138
20 p.p.m.	31	4.9	53	.01208	282

The data obtained in this study show that at low concentrations of iodine (1 p.p.m. as potassium iodide) the respiratory activity, as represented by weight of carbon dioxide given off per gram of fresh weight over a definite time, is lowered. This lowering occurs independently of whether the plants do or do not show visible toxic effects. From this point there is a gradual recovery of normal respiratory activity which is usually reached at iodine concentrations of 5 to 10 p.p.m. The

curve of the average in fig. 8 indicates that recovery occurs between 1 and 5 p.p.m., but this is because the average includes the erratic value represented in fig. 2 of determination 1. These results agree in general with those of Scharrer and Claus reported above.

Definitely *visible* toxic effects are evident only at the two higher concentrations, and these are the cultures showing the great increase in respiration. It should be noted, however, that

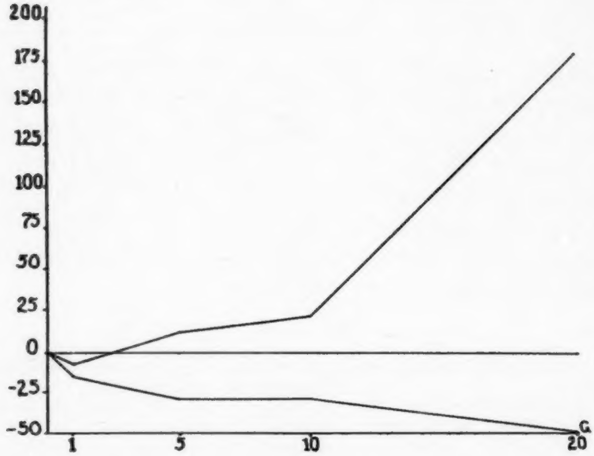


Fig. 8. Average of all respiration determinations.

the extent of the increase is not proportional to the degree of visible injury, and in some instances plants apparently healthy (although often not) show remarkable respiratory stimulation. It is also to be noted that this great increase, sometimes more than 300 per cent for the 20 p.p.m. plants, over that of the controls is a permanent respiratory level and is not the usual respiratory stimulation by toxic substances reported by many authors, which is of a temporary nature.

Copeland ('03) found a close parallel between the toxicity of various metal ions and the expulsion of carbon dioxide from the cell. He points out that this may be due to the power of metallic ions to cause the decomposition of carbonate in the

cell sap, and not therefore a stimulation of respiration. This interpretation is supported by the fact that these ions also cause an expulsion of carbon dioxide from tap water. Iodine and antipyrin did not evolve carbon dioxide from tap water, and therefore these substances may be assumed to stimulate the carbon dioxide evolution by an action on the protoplasm itself. This author mentioned that no poison has been found that does not stimulate carbon-dioxide production.

Irving ('11) reported that the proper concentration of chloroform would stimulate respiration of barley shoots and cherry laurel. Thoday ('13) also found that chloroform stimulated respiration and moreover that the absorption of oxygen kept apace with the increased carbon-dioxide production, and therefore he concluded that the respiratory process remained properly coordinated. This was found true only at the lower concentrations. A general review of the toxic substances stimulating respiration is too extended to include here, but attention should also be called to the work of Brooks ('18), Thomas ('18), and Ray ('23 a, b, c). There is general agreement that toxic substances stimulate carbon-dioxide evolution, and consequently we may not ascribe the great increase of carbon dioxide in our plants at the higher and obviously toxic concentrations as entirely due to any specific effect of iodine. However, the fact that the increase in respiration did not parallel the extent of injury does show that the iodine has some effect aside from the general stimulation by toxic substances. Whether our results conflict with those of Stoklasa cannot be decided at this time. He reports that the sugar beets used by him were stimulated in growth as well as in the respiratory activity of the roots, and if this be true the question of toxicity would not be a factor. It is entirely possible that different species of plants react differently.

E. OXIDIZING ENZYMES

1. *Catalase.*—Issajew ('05) studied the effect of hydriodic acid, potassium iodide, and of elementary iodine on preparations of yeast catalase. A concentration of N/2000 hydriodic acid completely inhibited catalase, while potassium iodide at a

concentration of N/10 depressed the reaction very slightly, if at all. Elementary iodine was completely inhibitory in a concentration of N/1152.

Juschtschenko ('11) removed the thyroid gland of dogs, and found that the blood catalase decreased, but if thyroid preparations were fed "per os" there was a recovery. It was found even possible to increase the catalase of the blood above the normal. The feeding of thyroid preparations to normal dogs, producing artificial hyperthyroidism, always caused an increase in blood catalase. When this feeding was discontinued, a normal condition was soon gained. Approximately the same results were obtained with rabbits. It is important to note that this author did not continue his observation of catalase activity in his test animals for a long enough time to show the effects of the accumulating amounts of iodine.

Strauss ('12) administered potassium iodide and sodium iodide to rabbits in amounts up to 4.0 grams per kilogram of body weight. The catalase activity was measured after 1, 4½, and 6 hours. The larger doses increased the catalase activity of the blood. This increase was ascribed to the effect of the iodides on the physiology of the organism rather than to any direct effect of these compounds in the blood.

Bach and Cheraskowa ('24) observed the activity of blood catalase of goats, 5 of which were normal animals and 7 had undergone thyroidectomy. Measurements were made daily for 10 days, but no difference between the two groups of animals was apparent.

The most extended study of this kind is that of Timofejewa ('27). *In vitro* studies of rabbits' blood showed that additions of potassium iodide were without effect, while a solution of iodine as potassium iodide, even at very low concentrations, lowered the catalase activity. Artificially prepared iodized proteins also exerted a depressing effect. Experiments with commercial thyroid were also performed. Subcutaneous injections of potassium iodide alone and of iodine dissolved in potassium iodide were given daily, and the blood samples were examined every 3 or 4 days. Both types of solutions showed

a preliminary depression extending over a period of about 3 months, but this period was followed by a great increase in activity. Thyroid feeding also gave a curve of catalase activity similar to that of inorganic iodine except that the preliminary period of depression was of much shorter duration. This author concludes, from a comparison of the *in vitro* and *in vivo* experiments, that there is a relation between the thyroid function and the activity of blood catalase.

Although the function of catalase, particularly its connection with respiratory metabolism, has not been definitely determined, its very wide distribution in plant and animal tissues postulates its great importance in living processes.

In view of the results of the animal physiologists cited above, it seemed important to carry out *in vivo* experiments in the present study. The plants used were of the same series as those used for the respiration experiments. Catalase was determined on May 1, 1932, after the plants had been growing in the respective solutions for 37 days, by the potassium permanganate method (Waksman and Davison, '26). The upper half of the plant was cut into small pieces and ground to a paste with purified quartz sand. A pinch of powdered calcium carbonate was added to neutralize the organic acids. The paste was then squeezed through two thicknesses of cheesecloth, and 1 cc. of the liquid was added to the substate and allowed to incubate at room temperature for 3 hours.

TABLE X

CATALASE DETERMINATION 1

Iodine concent. (p.p.m.)	Fresh wt. of tops (gms.)	Fresh wt. (control as 100)	H_2O_2 decomposition of 1 cc. of sap			
			No buffer		With buffer	
			Equivalents of H_2O_2 used up	Control as 100	Equivalents of H_2O_2 used up	Control of "no buffer" as 100
Control	19.8	100	.005336	100	.006628	124
1	9.6	.48	.005306	99	.005952	112
5	9.4	.47	.004502	84	.005890	110
10	6.5	.33	.005888	110	.006304	118
20	6.0	.30	.007060	149	.008530	159

TABLE XI

CATALASE DETERMINATION 2

Iodine concent. (p.p.m.)	Fresh wt. of tops (gms.)	Fresh wt. (control as 100)	H_2O_2 decomposition of 1 cc. of sap			
			No buffer		With buffer	
			Equivalents of H_2O_2 used up	Control as 100	Equivalents of H_2O_2 used up	Control of "no buffer" as 100
Control	10.7	100	.006424	100	.007250	113
1	6.6	62	.003700	58	.002768	43
5	9.7	91	.005090	79	.005770	89
10	7.4	69	.005638	88	.006916	108
20	10.4	101	.007444	116	.008096	126

TABLE XII

CATALASE DETERMINATION 3

Iodine concent. (p.p.m.)	Fresh wt. of tops (gms.)	Fresh wt. (control as 100)	H_2O_2 decomposition by 1 cc. of sap			
			No buffer		With buffer	
			Equivalents of H_2O_2 used up	Control as 100	Equivalents of H_2O_2 used up	Control of "no buffer" as 100
Control	10.2	100	.006420	100	.006940	108
1	5.6	55	.004880	76	.005760	89
5	6.0	59	.005658	88	.006454	101
10	7.4	73	.008778	137	.009542	149
20	9.4	92	.007700	119	.008918	139

TABLE XIII

CATALASE DETERMINATION 4

Iodine concent. (p.p.m.)	Fresh wt. of tops (gms.)	Fresh wt. (control as 100)	H_2O_2 decomposed by 1 cc. of sap			
			No buffer		With buffer	
			Equivalents of H_2O_2 used up	Control as 100	Equivalents of H_2O_2 used up	Control of "no buffer" as 100
Control	10.0	100	.007098	100	.007894	111
1	19.1	191	.005046	71	.006012	85
5	8.4	84	.005706	83	.006610	93
10	8.0	80	.006732	95	.007730	109
20	8.3	83	.008084	113	.091600	129

Parallel determinations were made on two types of substrate, one being buffered at pH 7.0, the other with no buffer. The buffered substrate was prepared by adding .0100 equivalent of hydrogen peroxide to 40 cc. of distilled water, then 5 cc. of the buffer solution. The buffer consisted of 11.6 grams of potassium-di-hydrogen-phosphate and 24.9 grams of di-sodium-hydrogen-phosphate per liter. Five-cc. portions added to 45 cc. of the reacting mixture maintains a pH of about 7.0. The unbuffered substrate was the same except for the absence of the buffer. The reacting substances were shaken every few

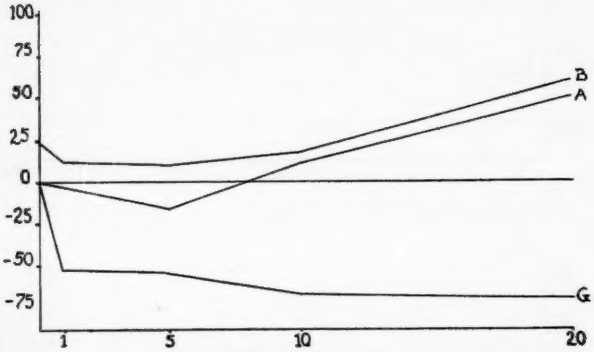


Fig. 9. Catalase Determination 1. A represents determinations on normal press juice; B, those in buffer.

TABLE XIV

AVERAGE OF ALL CATALASE DETERMINATIONS

Iodine concent. (p.p.m.)	Fresh wt. of tops (gms.)	Fresh wt. (control as 100)	H_2O_2 decomposed by 1 cc. of sap			
			No buffer		With buffer	
			Equivalents of H_2O_2 used up	Control as 100	Equivalents of H_2O_2 used up	Control of "no buffer" as 100
Control	12.6	100	.006319	100	.007178	114
1	10.2	81	.004733	76	.005123	82
5	8.4	67	.005239	83	.006181	98
10	7.3	58	.006759	108	.007623	121
20	8.5	68	.007547	124	.008676	138

minutes to insure thorough mixing. At the desired time, the reaction was stopped by adding 20 cc. of a 25 per cent sulphuric acid solution. Titration was carried out immediately with N/5 potassium permanganate.

The results (tables X-XIV and figs. 9–13 inclusive) show that the plants growing in the lower concentrations of potassium iodide exhibited a lower catalase activity of the sap than did

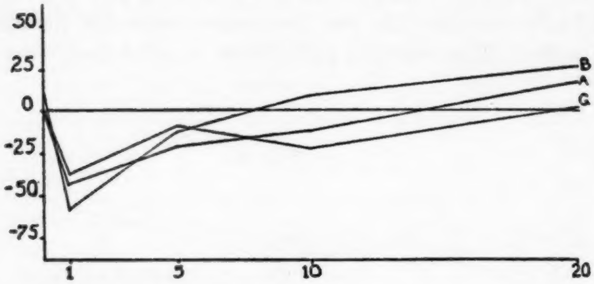


Fig. 10. Catalase Determination 2.

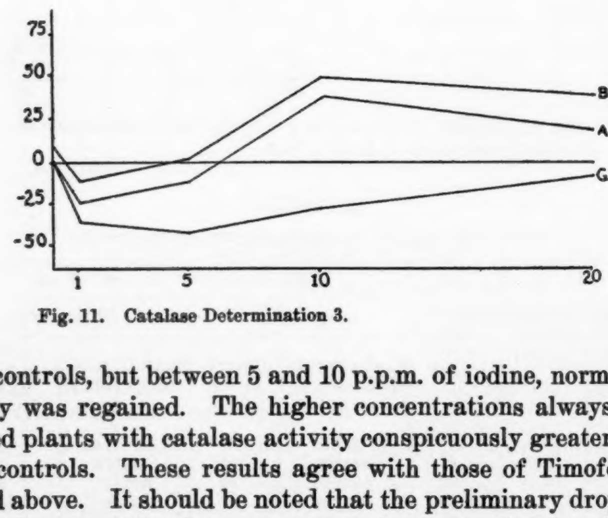


Fig. 11. Catalase Determination 3.

the controls, but between 5 and 10 p.p.m. of iodine, normal activity was regained. The higher concentrations always produced plants with catalase activity conspicuously greater than the controls. These results agree with those of Timofejewa cited above. It should be noted that the preliminary drop and the subsequent rise does not seem to be proportional to the degree of iodine injury as shown by the fresh weight of tops.

This is particularly noticeable in fig. 12, which shows the plant growing in 1 p.p.m. iodine as potassium iodide to be much larger than the control, yet this exceptional plant exhibited the preliminary drop just as did all the other plants growing in this type of solution.

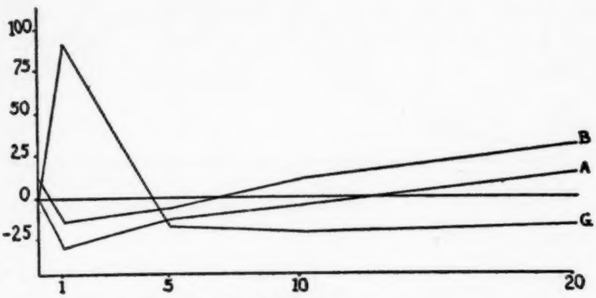


Fig. 12. Catalase Determination 4.

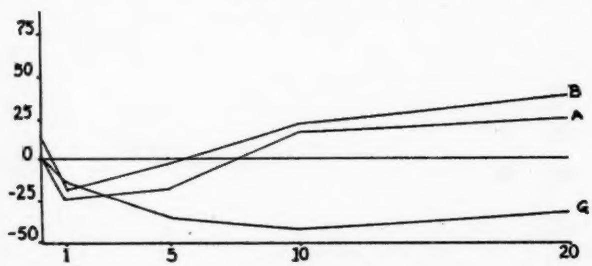


Fig. 13. Average of all catalase determinations.

2. *Peroxidase.*—The method of Guthrie ('31) was used to determine quantitatively peroxidase and oxidase. This author points out that the mixture of alpha-naphthol and paraphenylenediamine is oxidized far too rapidly for quantitative determination of these enzymes if the mixture is near neutrality, but if the reacting solution be buffered at pH 4.5, the rate of oxidation is sufficiently slow to allow accurate comparisons to be made. This acidity also inhibits catalase and prevents the interference of this enzyme.

The citrate buffer was made by dissolving 21 grams of

crystalline citric acid in 170 cc. of normal sodium hydroxide, and diluting to 1 liter. The substrate was prepared as follows: to 200 cc. of the citrate buffer, 200 cc. of water was

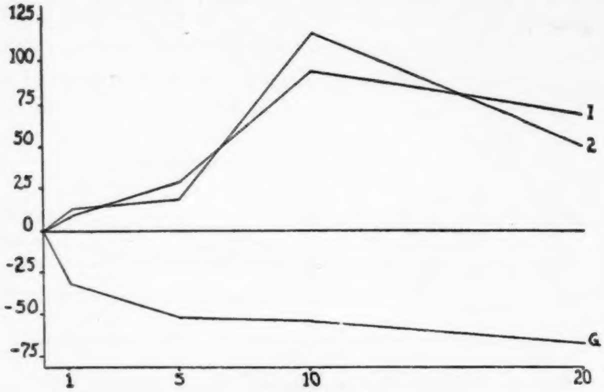


Fig. 14. Peroxidase Determination 1.

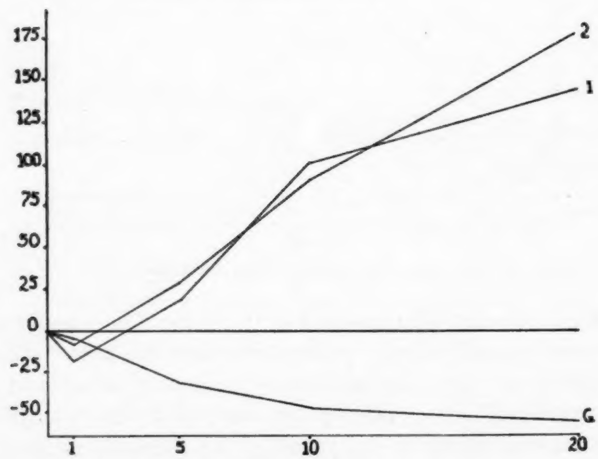


Fig. 15. Peroxidase Determination 2.

added, and then 1 gram of para-phenylenediamine hydrochloride and 20 cc. of a 4 per cent solution of alpha-naphthol in 50 per cent alcohol. This was filtered and used immediately.

The upper portions of the plants were ground with purified quartz sand, and the mass squeezed through cheese-

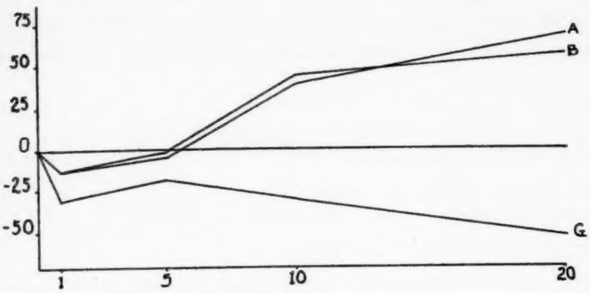


Fig. 16. Peroxidase Determination 3.

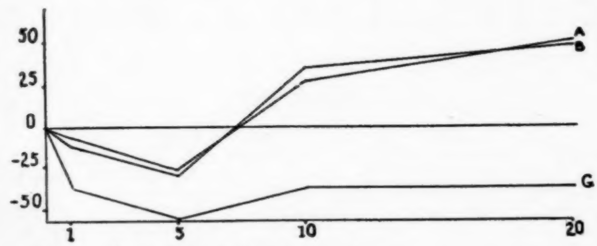


Fig. 17. Peroxidase Determination 4.

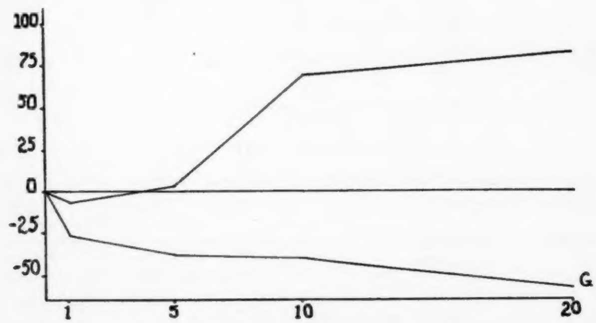


Fig. 18. Average of all peroxidase determinations.

cloth. Portions of 1 cc. were added to 25 cc. of the freshly prepared substrate. This was followed by the addition of 5 cc.

of N/20 hydrogen peroxide. After 15 minutes the reaction was stopped by the addition of 5 cc. of a .1 per cent aqueous solution of potassium cyanide. Twenty-five cc. of toluene were then added and shaken vigorously until the water layer appeared colorless. The toluene, containing the indophenol,

TABLE XV

PEROXIDASE DETERMINATION 1

Iodine concent. (p.p.m.)	Fresh wt. of tops (gms.)	Fresh wt. (control as 100)	Relative peroxidase activity	
			Determin. A (control as 100)	Determin. B (control as 100)
Control	27.7	100	100	100
1	18.2	69	109	114
5	13.5	49	128	119
10	12.7	46	194	217
20	9.1	33	167	149

TABLE XVI

PEROXIDASE DETERMINATION 2

Iodine concent. (p.p.m.)	Fresh wt. of tops (gms.)	Fresh wt. (control as 100)	Relative peroxidase activity	
			Determin. A (control as 100)	Determin. B (control as 100)
Control	18.3	100	100	100
1	17.5	95	83	92
5	12.6	69	118	127
10	10.7	53	200	189
20	8.1	44	244	279

TABLE XVII

PEROXIDASE DETERMINATION 3

Iodine concent. (p.p.m.)	Fresh wt. of tops (gms.)	Fresh wt. (control as 100)	Relative peroxidase activity	
			Determin. A (control as 100)	Determin. B (control as 100)
Control	17.9	100	100	100
1	12.5	69	89	89
5	14.7	82	96	98
10	12.6	71	139	143
20	6.1	48	167	156

TABLE XVIII

PEROXIDASE DETERMINATION 4

Iodine Concent. (p.p.m.)	Fresh wt. of tops (gms.)	Fresh wt. (control as 100)	Relative peroxidase activity	
			Determin. A (control as 100)	Determin. B (control as 100)
Control	17.6	100	100	100
1	11.3	64	94	87
5	7.7	44	74	72
10	11.2	64	127	135
20	7.2	41	149	147

TABLE XIX

AVERAGE OF ALL PEROXIDASE DETERMINATIONS

Iodine concent. (p.p.m.)	Fresh wt. of tops (gms.)	Fresh wt. (control as 100)	Relative peroxidase activity (control as 100)
Control	20.4	100	100
1	14.9	74	95
5	12.1	61	105
10	11.8	59	168
20	7.6	42	182

was then poured off, centrifuged, and examined in a colorimeter. Samples from the entire series of plants were run simultaneously and in duplicate.

The oxidase determinations were carried out identically and simultaneously on the same samples of press juice, but with the omission of the hydrogen peroxide.

Since comparative results, rather than absolute amounts, were the object of the study, the intensity of color of the centrifuged toluene-indophenol solution obtained from the action of the sap of the control plants was taken as 100, and the other values calculated comparatively. The determinations were made on May 8, 1932, after the plants had grown in the respective solutions for 44 days.

The results are set forth in tables XV to XIX, and figs. 14 to 18 inclusive. Here again there is a preliminary period of depression, followed by a significant increase. A comparison of the growth curves with the enzyme curves shows that the

increase of peroxidase activity is not proportional to the degree of injury by the toxic concentrations of potassium iodide. The curves do, however, closely parallel those of respiration.

3. *Oxygenase.*—The terms oxidase and oxygenase have been variously used in the literature. Bach and Chodat defined oxygenase as an organic peroxide. Kostytschew ('26) states that, "Die unbeständigen Substanzen vom Peroxydtypus führen den Namen Oxygenasen. Ihre chemische Natur ist bis jetzt unbekannt, doch sind sie allem Anschein nach organische Substanzen. Die reduzierten Oxygenasen, die den labilen Saurerstoff abgespalten haben, nennt man Oxydasen. Die Oxydasen gehen unter Sauerstoffaufnahme wieder in Oxygenasen über." Other authors have used oxidase to denote the oxygen-activating enzyme and oxygenase as the complex of oxidase plus the quinone-like substances produced by it. This complex has the ability to oxidize many substances and has therefore been loosely referred to as oxygenase. In the present work, the term oxygenase is defined as the enzymatic catalyst by which molecular oxygen is activated toward substances of the general catechol type.

As indicated above, the determinations of oxygenase activity were carried out like those of peroxidase except that hydrogen peroxide was omitted from the reaction mixture. Theoretically, this should be a satisfactory means of determining oxidase activity, but as has been pointed out by Onslow ('31), the problem is complicated as shown in the following paragraphs.

Szent-Györgyi ('25), in his study of oxidation mechanisms of the potato, determined that compounds of the ortho-quinone type were formed by the action of oxygenase on compounds having the ortho-dihydroxy-benzene grouping. These orthoquinones are to be considered as the final product of the action of oxygenase itself. During this process, peroxide is formed: (1) either by the preliminary formation of organic (catechol) peroxides by a union with free oxygen of the air, and the subsequent breaking up to give hydrogen peroxide and some oxidation product of catechol; or (2) more directly by the

transference of hydrogen from catechol compounds to molecular oxygen producing hydrogen peroxide and orthoquinone.

In the present oxygenase studies 1-cc. aliquots of the same expressed plant juice as used for the peroxidase determination were taken, and both enzymes determined simultaneously. Since peroxidase was present in large amounts, particularly in the plants grown in the higher potassium-iodide concentrations, there is no reason why the peroxide formed by oxygenase could not have initiated reactions giving the same oxidation products as the oxygenase. This is undoubtedly a source of error in the present work, since a part of the color developed (indo-phenol) came from this side reaction between peroxidase and the developing peroxide. If, however, these reactions seriously affected the results, we could expect the plant juice having the greatest peroxidase to give also the greatest apparent oxygenase reaction. From a comparison of the curves for these two enzymes it may be seen that this is not the case. The significance of this source of error is further lessened by the fact that some of the peroxide acted upon by peroxidase originated from the oxygenase reactions. For this reason it is seen that while more indo-phenol may have been formed, the increase was proportional to that of oxygenase activity, and the general shape of the curve would be the same.

The results of the present study are set forth in tables xx–xxiv, and figs. 19–23. While the individual curves vary considerably the average (fig. 23) shows a small but progressive decrease of oxygenase activity in the plants grown in the higher potassium iodide solutions. This is particularly significant since the source of error discussed above tends to push the curve up, rather than down. It should be emphasized again that aliquots of these *same samples* of press juice show simultaneously a great peroxidase increase.

Owing to the comparatively slight variation of the amount of oxygenase activity the question might be raised as to whether this enzyme was present at all, especially since no absolute values are given. Onslow ('21) has determined that

only about 60 per cent of the higher green plants contain the catechol-oxygenase system. Tomato fruit is known, moreover, to contain no oxygenase. In the controls of the reagents alone, carried out simultaneously with the oxygenase determination, a little indo-phenol was developed through atmospheric oxidation, but in no case did this amount ever exceed 16 per cent of that found in the experimental solutions. Hence the presence of oxygenase in the vegetative portion is clearly

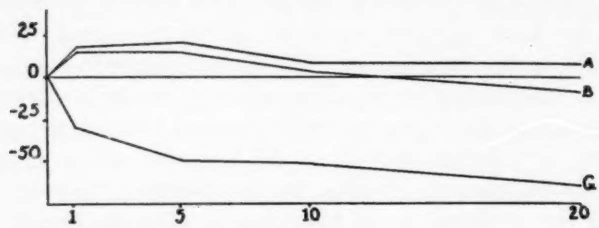


Fig. 19. Oxygenase Determination 1.

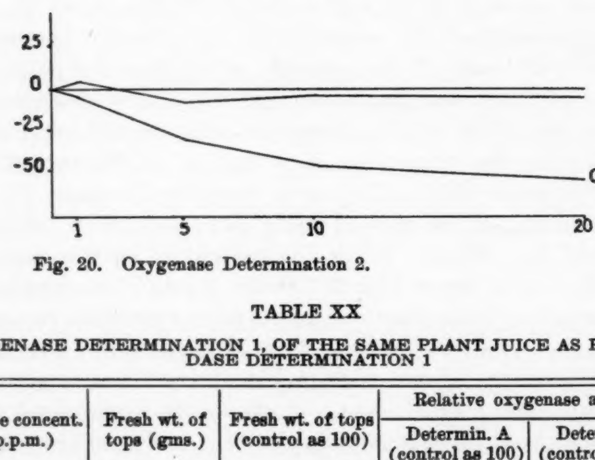


Fig. 20. Oxygenase Determination 2.

TABLE XX

OXYGENASE DETERMINATION 1, OF THE SAME PLANT JUICE AS PEROXIDASE DETERMINATION 1

Iodine concent. (p.p.m.)	Fresh wt. of tops (gms.)	Fresh wt. of tops (control as 100)	Relative oxygenase activity	
			Determin. A (control as 100)	Determin. B (control as 100)
Control	27.7	100	100	100
1	18.2	69	118	115
5	13.5	49	121	115
10	12.7	46	108	102
20	9.1	33	107	91

TABLE XXI

OXYGENASE DETERMINATION 2, OF THE SAME PLANT JUICE AS PEROXIDASE DETERMINATION 2

Iodine concent. (p.p.m.)	Fresh wt. of tops (gms.)	Fresh wt. of tops (control as 100)	Relative oxygenase activity	
			Determin. A (control as 100)	Determin. B (control as 100)
Control	18.3	100	100	Determin. lost
1	17.5	95	104	Determin. lost
5	12.6	69	92	Determin. lost
10	10.7	53	97	Determin. lost
20	8.1	44	94	Determin. lost

TABLE XXII

OXYGENASE DETERMINATION 3, OF THE SAME PLANT JUICE AS PEROXIDASE DETERMINATION 3

Iodine concent. (p.p.m.)	Fresh wt. of tops (gms.)	Fresh wt. of tops (control as 100)	Relative oxygenase activity	
			Determin. A (control as 100)	Determin. B (control as 100)
Control	17.9	100	100	100
1	12.5	69	80	83
5	14.7	82	80	81
10	12.6	71	77	82
20	6.1	48	68	79

TABLE XXIII

OXYGENASE DETERMINATION 4, OF THE SAME PLANT JUICE AS PEROXIDASE DETERMINATION 4

Iodine concent. (p.p.m.)	Fresh wt. of tops (gms.)	Fresh wt. of tops (control as 100)	Relative oxygenase activity	
			Determin. A (control as 100)	Determin. B (control as 100)
Control	17.6	100	100	100
1	11.3	64	63	91
5	7.7	44	56	71
10	11.2	64	68	92
20	7.2	41	57	73

demonstrated. Bunzell ('16) also found oxygenase activity in the tomato plant. Two cc. of juice expressed from the leaves absorbed sufficient atmospheric oxygen to reduce the

TABLE XXIV

AVERAGE OF ALL OXYGENASE DETERMINATIONS

Iodine concent. (p.p.m.)	Fresh wt. of tops (gms.)	Fresh wt. of tops (control as 100)	Relative oxygenase activity (control as 100)
Control	20.4	100	100
1	14.9	74	93
5	12.1	61	88
10	11.8	59	89
20	7.6	42	56

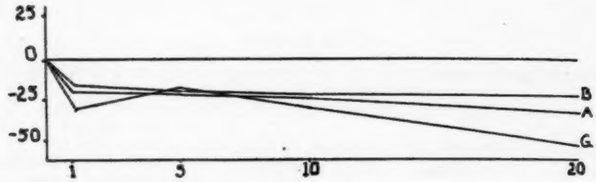


Fig. 21. Oxygenase Determination 3.

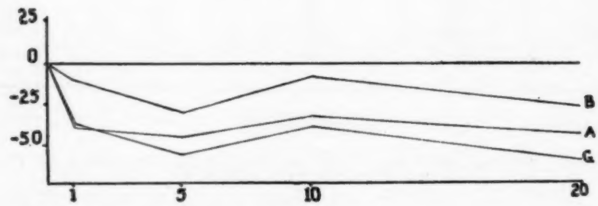


Fig. 22. Oxygenase Determination 4.

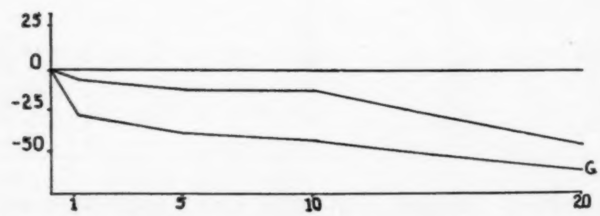


Fig. 23. Average of all oxygenase determinations.

pressure in his apparatus 1.10–1.60 mm. of mercury. The substrate used was of the catechol type, and could therefore give the indo-phenol reaction by the intermediate formation of quinones.

The significance of the oxygenase-catechol system in plants has been much discussed. Palladin's theory, that respiratory chromogens, e.g., catechol-oxygenase or similar systems, furnish a mechanism for respiratory oxidation, depends upon this enzyme. This position is materially weakened by the fact that in the tissues of only about one-half of the higher plant orders may such a system be recognized. However, Keilin ('29) held the respiratory function of such an oxidation mechanism to be conclusively proved for yeast and animal tissues. This author used the same reagents and approximately the same procedure that was used in the present work.

Onslow ('31) believes that an effective mechanism would be more universally distributed in tissues, and therefore the existence of the catechol-oxygenase system should be regarded as aside from the fundamental respiratory mechanism. This author also emphasized experimental results, which show that only plants containing the substrate, e.g., catechol or similar compounds, contain oxygenase; hence the real problem is that of the physiology of the formation of these substances rather than their connection with oxygenase.

A comparison of the respiratory with the oxygenase curves would indicate that there is no connection in the tomato plant between respiration activity as shown by carbon-dioxide output and this enzyme. On the other hand, the peroxidase curves do show such a relationship. The author therefore believes it logical to assume that respiration is being carried on in some manner entirely separate from the catechol-oxygenase system. It then follows that if the comparatively small decrease of oxygenase activity in the plants grown in solutions of higher concentrations of potassium iodide be considered significant, it must be assumed that the increasing iodine content of the tissue has interfered with the normal physiology of the production of the ortho-dihydroxy-benzene

type of compounds. It is greatly regretted that the lack of sufficient tissue prevented an actual quantitative study of these substances.

F. NON-OXIDIZING ENZYMES

1. Invertase.—The effect of iodine and potassium iodide on the activity of invertase has been carefully studied *in vitro.* Euler and Landergren ('22) were the first to attack this phase of iodine physiology. They added a solution containing .74 mgm. of elemental iodine and 1.50 mgms. of potassium iodide per cc. to their invertase preparation and held it at 17° C. for one hour. The preparation was then added to the sugar solution containing 4.8 gms. of sucrose and 2 per cent phosphate, giving a total volume of 60 cc. These authors found that the concentration of potassium iodide used (.01 M) did not materially inactivate the invertase. The relative inversion velocity was 58.7 per cent of the control when .07 mgm. of elemental iodine was present. The relative inversion velocity was 45.9 per cent of the control in the presence of 10 times (.74 mgm.) this amount of iodine, thus the decrease of invertase activity was small compared to the wide range of iodine concentrations. It was concluded that while invertase was sensitive to iodine, most of its activity remained stable, even with large amounts of this substance. Treatment with sodium-thio-sulphate did not reactivate the enzyme.

The work was continued by Euler and Josephson ('22, '23). They considered that the invertase formed an iodine-invertase compound and they attempted its isolation and also to complete its inactivation by a silver salt. They found that the degree of inactivation by iodine and potassium iodide mixture was dependent on the time during which the iodine acts on the enzyme, and called this the "incubation" time. Complete inactivation by iodine was accomplished by lengthening the period of incubation. It is not yet certain that the inactivation depends upon any stoichiometric relation. There is also the possibility that iodine catalyzes the inactivation and therefore does not appear as an end product. Hence it might in-

activate a larger number of invertase molecules than would correspond to its own number of molecules. Their study of the reaction constant of the inactivation process tends to support this assumption. These workers also found that while increasing amounts of iodine did not inactivate the invertase beyond a certain point, if the incubation time were short, bromine water could obviously call forth any degree of inactivation more or less independent of the incubation time, the degree of inactivation depending upon the bromine content. In summing up their work, Euler and Josephson made the following statements concerning the relation of invertase to iodine:

1. Iodine poisoning is dependent upon the time of incubation.
2. Iodine appears to exert a stronger effect on invertase of high purity than does bromine.
3. One gram of iodine suffices, under the conditions used, to depress the activity of 20,000 grams of invertase, of an initial inversion power of If=230, to about one-half.
4. The 50 per cent inhibited enzymes show the same optimal pH as the control.
5. The 50 per cent inhibited enzyme is held to be an iodine-invertase compound which is also an enzyme but of lower inversion power.
6. Bromine and silver agree very closely in their ability to inactivate invertase, but do not agree with the iodine inactivation equivalent. This indicates a similarity of the reaction of invertase to silver and bromine, but a specific and different reaction to iodine.

The present investigation of the effect of potassium iodide upon the invertase activity was carried out *in vivo*. Tomato plants grown in nutrient solutions containing increasing amounts of potassium iodide were harvested after a growth period of 45 days. The tops were cut in small pieces and dried in absolute alcohol and washed in ether and then spread out to dry. The dried material was then ground to a fine powder. One gram of the dry powder was mixed with 100 cc. of distilled water and soaked 12 hours in the Kelvinator. Then 15 grams of commercial sucrose were added and the mixture incubated at 37° C. for 24–48 hours. After incubation, the mixture was filtered and 10-cc. aliquots analyzed for reducing sugars. To the 10-cc. aliquots 15 cc. distilled water and 30 cc. of Fehling's solution were added. The 5 solutions to be com-

pared were simultaneously boiled 3 minutes in large test-tubes in the same beaker of water. This insured comparable conditions of reduction in the different types of plants, and since

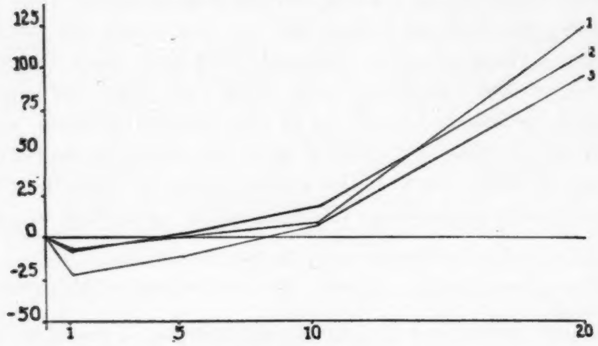


Fig. 24. Invertase Determination 1.

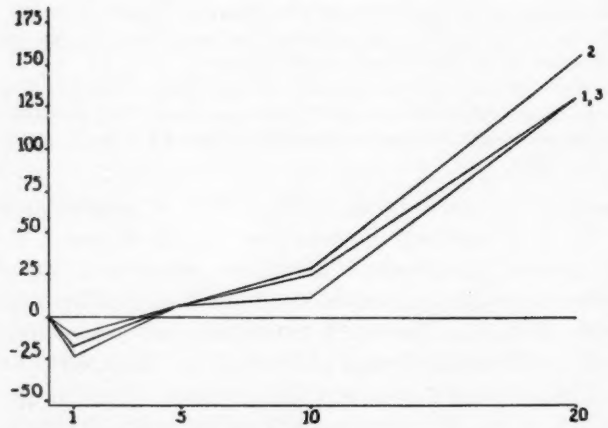


Fig. 25. Invertase Determination 2.

the final values are reported as per cent of the control, the absolute amount of reduction in any one series becomes unimportant. The copper oxide was immediately filtered off and dissolved in Bertrand's solution (20 per cent sulphuric acid which had been saturated with ferric-sulphate) as recom-

mended by Morris and Wesp ('32). The amount of reduced iron in the acid solution was titrated with N/20 potassium permanganate.

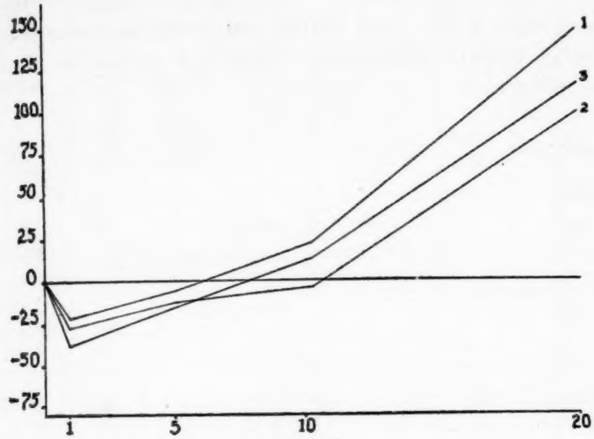


Fig. 26. Invertase Determination 3.

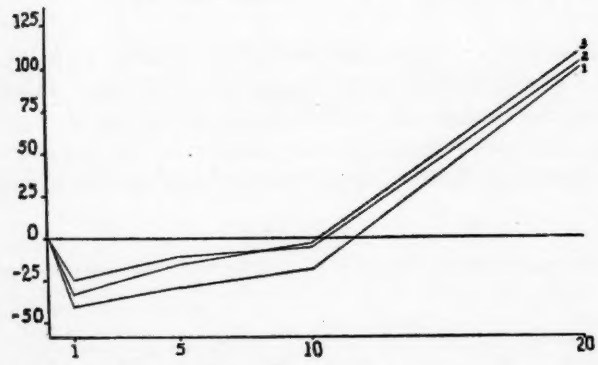


Fig. 27. Invertase Determination 4.

The number of cubic centimeters of permanganate used was taken as the basis for calculating the results comparatively. The results are shown in tables XXV–XXIX and in figs. 24–28. As is the case with the previous enzymes studied, with the ex-

ception of oxygenase, the preliminary period of depression is followed by a great increase. The effect of iodine in the previous *in vitro* studies is seen to be very different from the present *in vivo* experiments. This further supports the findings of Fuller ('32), who stated that enzymes behaved very differently toward ultra-violet radiation in his *in vivo* and *in vitro* studies.

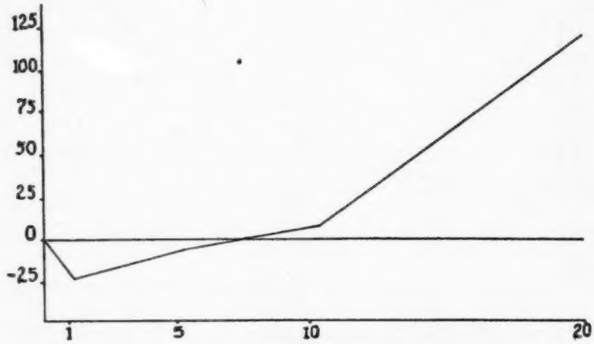


Fig. 28. Average of all invertase determinations.

Onslow ('31) reviews the literature dealing with the substrate for respiration in the higher green plants. In studies made on the change of carbohydrate fractions of respiring organs she noted that a hexose sugar is the substrate for respiration, and further, that fructose from sucrose hydrolysis

TABLE XXV

INVERTASE DETERMINATION 1, INCUBATION PERIOD 24 HOURS

Iodine concent. in p.p.m.	cc. $KMnO_4$ used				Relative amt. $KMnO_4$ used (control as 100)			
	Set 1	Set 2	Set 3	Ave.	Set 1	Set 2	Set 3	Ave.
Control	7.61	9.17	8.58	8.45	100	100	100	100
1	6.98	8.56	6.73	7.42	92	93	78	88
5	7.67	9.22	7.70	8.20	101	101	89	97
10	8.22	10.82	9.15	9.40	108	118	107	111
20	17.20	19.16	17.02	17.79	226	209	198	210

TABLE XXVI

INVERTASE DETERMINATION 2, INCUBATION PERIOD 24 HOURS

Iodine concent. in p.p.m.	cc. $KMnO_4$ used				Relative amt. $KMnO_4$ used (control as 100)			
	Set 1	Set 2	Set 3	Ave.	Set 1	Set 2	Set 3	Ave.
Control	10.92	10.45	9.22	10.20	100	100	100	100
1	8.30	8.54	8.25	8.36	76	82	89	82
5	11.66	11.21	9.92	10.93	107	107	107	107
10	12.07	13.16	12.05	12.43	111	125	129	122
20	25.57	26.72	21.61	24.63	234	255	234	241

TABLE XXVII

INVERTASE DETERMINATION 3, INCUBATION PERIOD 40 HOURS

Iodine concent. in p.p.m.	cc. $KMnO_4$ used				Relative amt. $KMnO_4$ used (control as 100)			
	Set 1	Set 2	Set 3	Ave.	Set 1	Set 2	Set 3	Ave.
Control	12.28	17.27	14.46	14.67	100	100	100	100
1	9.46	12.59	9.05	10.37	77	73	62	71
5	11.71	15.04	12.54	13.10	95	87	86	89
10	15.08	16.67	16.72	16.16	123	97	115	112
20	30.89	35.12	31.68	32.56	251	203	219	224

TABLE XXVIII

INVERTASE DETERMINATION 4, INCUBATION PERIOD 48 HOURS

Iodine concent. in p.p.m.	cc. $KMnO_4$ used				Relative amt. $KMnO_4$ used (control as 100)			
	Set 1	Set 2	Set 3	Ave.	Set 1	Set 2	Set 3	Ave.
Control	15.14	17.05	14.80	15.69	100	100	100	100
1	8.78	12.85	9.69	10.44	58	75	65	66
5	10.56	15.30	12.51	12.79	69	89	84	81
10	11.24	16.16	13.36	13.56	81	95	89	88
20	30.38	34.70	30.89	31.79	201	203	208	204

is preferentially used. Should this fructose be exhausted, the more stable glucose may be utilized. To quote only one of many available studies concerning this problem, Evans ('28) found 80 per cent of the total hexose in stored apples to be glucose, the fructose from disaccharide hydrolysis having been depleted through respiration.

TABLE XXIX

AVERAGE OF ALL INVERTASE DETERMINATIONS, THE CONTROL TAKEN AS 100

Iodine concent. in p.p.m.	Relative invertase activity
Control	100
1	77
5	94
10	108
20	220

Since it is well authenticated that a hexose sugar serves as the respiratory substrate, it is to be expected that a conspicuous increase in invertase activity would accompany accelerated respiration. This is based on the concept discussed later that sucrose normally predominates over hexose in the tomato plant. Wehmer ('31) summarizes the analytical results of many publications concerning the sugar content of the fruit. The total sugar has been variously reported as being 3–5 per cent of the fresh weight. Sucrose has been reported as being about 1.7 per cent, levulose 1.12 per cent and glucose 1.12 per cent. Unfortunately, no published analyses have come to our notice concerning the carbohydrate fractions of the vegetative tissue.

2. Peptase.—Peptase, being a plant enzyme not directly concerned with the respiratory process, was used in order to determine whether iodine would affect it differently from those involving respiratory metabolism. The method of procedure was that of Fisher ('19) The number of free carboxyl groups was estimated by the method of Sörensen ('08).

The plants were grown in the culture solutions for 43 days. The tops were then shredded and dried by alcohol and washed with acetone as described for the material used for the invertase determinations. One gm. of dry plant powder was added to 200 cc. of a 2 per cent Witte peptone solution, and the mixture plus a little toluol was incubated for 4 days at 37° C. After that time, the material was filtered and the residue washed until the filtrate came to 400 cc. The filtrate was de-

colorized by shaking with alumina cream. Aliquots of 40 cc. of the prepared solution were titrated in the following manner. First, 15 cc. of a formaldehyde mixture were added. This solution was made by mixing 50 cc. of commercial formalin (40 per cent) with 25 cc. of absolute alcohol, and then adding 10 cc. of indicator. The indicator was prepared by dissolving .50 gram of Grubler's thymol-phthalein in 1000 cc. of 93 per cent alcohol. The free amino groups of the liberated amino acids react as a base, thereby masking the carboxyl groups. Hence formaldehyde is used to neutralize those groups by the formation of methylene compounds. The free carboxyl groups were then titrated by N/5 barium hydroxide.

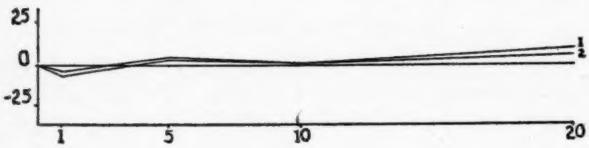


Fig. 29. Peptase determinations.

The number of cubic centimeters of hydroxide used is taken as the basis for comparison. The results are shown in tables XXX and XXXI and in fig. 29. The figure shows a very small preliminary drop, followed by a very small rise in peptoclastic activity. It is probable that these changes are not significant in view of possible inaccuracies of the method.

TABLE XXX

PEPTASE DETERMINATION 1

Iodine conc. in p.p.m.	cc. $Ba(OH)_2$ used				Comparative amount $Ba(OH)_2$ used (control as 100)			
	Titrat. 1	Titrat. 2	Titrat. 3	Ave.	Titrat. 1	Titrat. 2	Titrat. 3	Ave.
Control	10.5	10.7	10.7	10.6	100	100	100	100
1	10.0	9.9	10.1	10.0	95	92	94	94
5	10.8	11.0	10.9	10.9	103	104	102	103
10	10.3	10.7	10.7	10.6	98	100	100	100
20	11.5	11.7	11.7	11.6	109	109	109	109

TABLE XXXI

PEPTASE DETERMINATION 2

Iodine conc. in p.p.m.	cc. Ba(OH)$_2$ used				Comparative amount Ba(OH)$_2$ used (control as 100)			
	Titrat. 1	Titrat. 2	Titrat. 3	Ave.	Titrat. 1	Titrat. 2	Titrat. 3	Ave.
Control	11.4	10.4	10.2	10.7	100	100	100	100
1	10.7	10.0	9.9	10.3	94	96	97	96
5	10.9	11.2	11.3	11.1	96	108	111	104
10	11.0	10.6	10.5	10.7	97	102	103	100
20	11.2	11.0	11.5	11.2	98	106	112	105

IV. The Relationships Between Oxygenase, Peroxidase, Invertase, and Catalase

A. Respiratory Pigments

The theory of the function of respiratory pigments has been elaborated at some length by Palladin ('08–'12), Palladin and Lwow ('13), and by Palladin and Tolstoi ('13). These workers considered that respiratory pigments accept hydrogen from biologically oxidizable material and transfer it to the oxygen of the air, the pigments thereby becoming reduced to chromogens. This theory would regard biological oxidation as essentially a dehydrogenation process. Other students of respiratory mechanism have placed a varying degree of importance on this interpretation. Bach, Thunberg, and Wieland each regard the transfer of hydrogen of great significance. Several specific carriers of hydrogen have been identified from time to time. The glutathione of Hopkins ('21 a, b), the cytochrome of Keilin ('29), the hexuronic acid of Szent-Györgyi ('31), and the blue pigment found in *Bacillus pyocyaneus* by Friedheim ('31) are examples.

The fact that methylene blue has been found by many workers to stimulate metabolism is evidence of the importance of a dehydrogenating system for respiration. Warburg, Kubowitz and Christian ('30 a, b) have determined the mechanism by which methylene blue accelerates respiration. They interpret the reaction by arranging the following three equations:

Methylene blue + n-hemoglobin + nH_2O → leuco methylene blue + n-methemoglobin.
n-methemoglobin + glucose → n-hemoglobin + glucose oxidation products + nH_2O.
Leuco methylene blue + H_2O_2 → methylene blue + H_2O.

These authors did not determine whether the sugar entered the reaction as glucose, phosphoric esters, or as some hexose cleavage product. The final reaction by which the leuco compound is oxidized to the colored state is defined as a "balancing" reaction, running parallel to the cellular catalytic processes, but the actual oxidation of the carbohydrate is accomplished by hemin iron.

Eddy ('31) found that intravenous injections of methylene blue increased the oxygen uptake of the organism.

B. OBJECTIONS TO THE DEHYDROGENATION THEORY OF BIOLOGICAL OXIDATION

The chemical reactions by which hydrogen may be removed from oxidizable materials appear to depend upon the action of enzymes, at least in most instances. The relation between respirational reaction and enzymes has been seriously questioned by some workers. Engler and Herzog ('09) maintain that the importance of enzymes in biological respiration is greatly overrated.

The fact that oxygenase and peroxidase catalyze only the reactions which account for the formation of water by the union of atmospheric oxygen and derived hydrogen and do not break carbon chains, is a serious objection to a close association of these enzymes with respiration. At least this would tend to place the oxidizing enzymes in some subsidiary relation to a breaking of the carbon chain. Bertrand ('96), Portier ('97), and Porodko ('04) placed so much importance on the fact that oxidizing enzymes could not unite oxygen to carbon to form carbon dioxide that they denied that these enzymes could attack any important respirational substrate to be found in tissue.

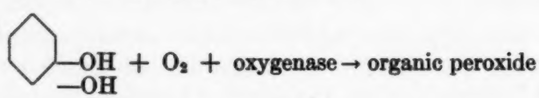
However, the case for oxidizing enzymes has been materially

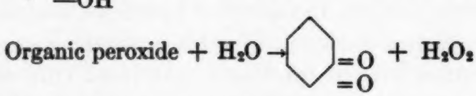
strengthened by the work of Lyon ('23, '27). This worker found that an aqueous extract of oxidase from potato tubers in the presence of sodium or potassium phosphate would slowly oxidize glucose or fructose with the liberation of carbon dioxide. The phosphate ion apparently acted as an intensifier of the oxidase. Wurmser ('32) aptly points out the probability of a dehydrogenating system coexisting with some system capable of breaking carbon chains.

C. CHEMICAL REACTIONS OF THE OXIDATION MECHANISM STUDIED IN THE PRESENT WORK

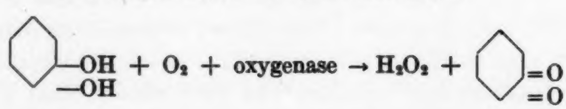
As pointed out above, the experimental substrate for the determinations of peroxidase and oxygenase consisted of a mixture of para-dimethyl-phenylene-diamine-hydrochloride and alpha-naphthol in an alcoholic solution. This differs from the original "Nadi" reagent in that it was buffered at a higher acidity to suppress the action of catalase and to slow down the rate of oxidation.

The steps of the reaction may be pictured as follows. The resulting quinone is to be considered as the final oxidation product of oxygenase itself. The reaction mixture may be assumed to contain compounds of the orthodihydroxy type since Onslow ('31) found these substances always present in plants exhibiting oxygenase activity.

1. a


b


Or the initial step may form H_2O_2 directly:

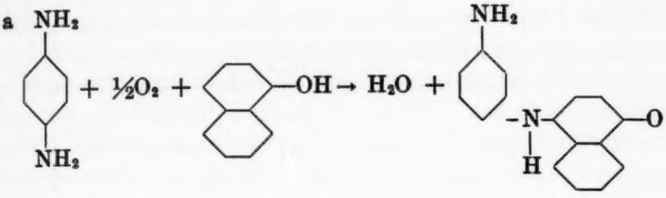
2.


The quinone reacts upon the artificial substrate, forming the colored product.

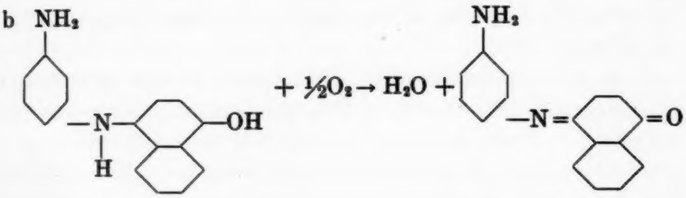
The indo-phenol may be considered as an artificial hydrogen acceptor. Such a compound in living tissue accepts hydrogen from the respirational substrate by the action of the dyhydrases.

Equations 3a, b, are those postulated by Rohmann and Spitzer ('95).

3. a

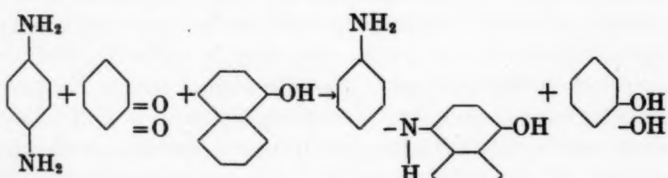


b

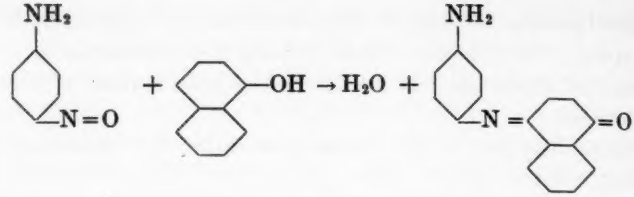


If quinone be considered as the final product of oxygenase, equation 3a would be modified as follows:

c



Since equation 3b is not dependent upon enzymatic catalysis, the leuco compound formed in 3c would autoxidize in the presence of atmospheric oxygen independently of quinone. It is not impossible that the para-phenylene-diamine might become oxidized to the nitroso stage, in which case it would condense with the α-naphthol to give the colored indo-phenol as follows:

4. 

Keilin ('29) distinguishes between his indo-phenol oxidase and the catechol oxidase of other workers. He describes indo-phenol oxidase as being insoluble, while catechol oxidase is soluble in water. They are both inhibited by potassium cyanide, hydrogen sulphide, and by carbon monoxide. Catechol oxidase is more sensitive to the inhibitory effect of carbon monoxide than indo-phenol oxidase. The catechol oxidase was described by him as having "all the essential properties of a typical oxidase."

It does not seem possible or necessary to separate these two oxidases in a discussion of the function of oxygen-activating enzymes in their relation to respiration. Any essential difference between these substances depends upon the assumption that indo-phenol oxidase oxidizes directly the aromatic amino groups of para-phenylene diamine or the leuco form of indo-phenol by the activation of atmospheric oxygen, while catechol oxidase acts only upon poly-hydroxy-benzene compounds, producing quinones, which then oxidize the leuco-indo-phenol or some naturally occurring chromogen. Keilin states that catechol oxidase will give the indo-phenol test in the presence of catechol compounds. According to Onslow ('31), this oxidase occurs only with the simultaneous occurrence of this substrate. Hence it is evident that the catechol oxidase in tissue preparations will always give the indo-phenol reaction unless great care has been used to remove all traces of polyphenolic compounds. On the other hand, in order to maintain the separate identity of these enzymes, it would be necessary to establish the fact that indo-phenol oxidase acts upon leuco indo-phenol or similar naturally occurring chromogens without the intermediate cooperation of such substances as the quinones.

This would be difficult to accomplish, since leuco indo-phenol is easily autoxidizable. It does not appear from an examination of the literature that such a demonstration has been made.

For demonstrating the oxidase of yeast cells, Keilin recommends a neutral 1 per cent solution of para-phenylene-diamine-hydrochloride, or the familiar "Nadi" reagent consisting of equal parts of M/100 solutions of di-methyl-para-phenylene-diamine-hydrochloride and alpha-naphthol in 50 per cent alcohol and 125 per cent aqueous sodium carbonate. These reagents by definition would demonstrate the presence of the indo-phenol oxidase. It is very important to note that Tolomei ('96), Grüss ('01), and Issajew ('04) also found an oxidizing enzyme in yeast by the use of various polyphenols. This oxidase by definition would be a catechol oxidase. Happold ('30) has studied the ability of bacteria to oxidize polyphenols and dimethyl-para-phenylene-diamine. Positive tests with these two reagents would correspond to Keilin's catechol oxidase and indo-phenol oxidase respectively. Positive oxidase reactions were found for eleven different bacteria. Of these, only *Staphylococcus albus* failed to give the oxidase reaction with catechol and the para-phenylene-diamine.

The work of Cook, Haldane and Mapson ('31) shows that it is entirely probable that different oxidases may exist in the cell. These workers studied the effect of carbon monoxide on the oxidation of various substances by *Bacillus Coli,* and concluded that the cell contains a number of oxidases differing from one another as do the different hemoglobins.

As pointed out above, Bunzell ('16) found an oxidase in tomato juice which acted upon pyrogallol and was therefore a catechol oxidase. In the present work the presence of an indo-phenol oxidase was determined by the use of the modified "Nadi" reagent. It is possible and probable that the catechol enzyme was the active catalyst in both cases.

The essential consideration in evaluating the work of Keilin and of other workers who postulate a great importance for the oxidases in respiration is that the hydrogen acceptor—the chromogens of Palladin, the respiration ferment of Warburg,

the cytochromes of Keilin, etc—is oxidized by their activity, and may then function again as a hydrogen acceptor. With this view, it is not necessary to separate the indo-phenol oxidase of Keilin and the catechol oxidase of other workers.

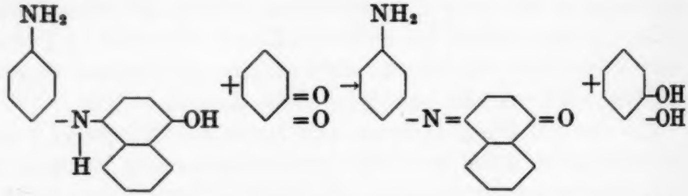
D. POSSIBLE ENZYMATIC SYSTEMS

Oxygenase-catechol-catalase.—Considering the indo-phenol as an artificial respiratory pigment we may arrange the following oxygenase-catechol-catalase system. In all reactions in which indo-phenol occurs, it is intended to figuratively represent all hydrogen acceptors which have been postulated by other workers and which are reversibly oxidized and reduced.

Oxygenase produces quinone and peroxide by equations 1 and 2.

The quinone restores the chromogen to the pigment. The power of quinones to accomplish this has been verified in the present work by *in vitro* experiments:

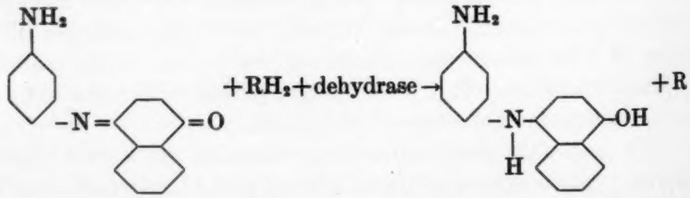
5.



The catechol compound is restored to quinone by equations 1 and 2.

The pigment accepts hydrogen from the respirational substrate (RH_2) by the action of dehydrases:

6.

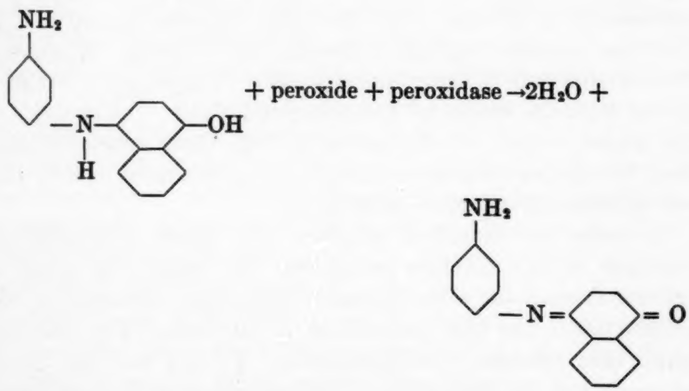


The above system would be theoretically self-perpetuating. The toxic effect of the accumulating hydrogen peroxide could

be prevented by catalase. In such a case catalase would be intimately associated with the respirational process and might be expected to fluctuate more or less proportionally with it.

Zaleski and Rosenberg ('11), Appleman ('18), Crocker and Harrington ('18), Burge ('20), and many others have sought to establish a relationship between this enzyme and respiration. Since it is assumed that catalase releases only molecular oxygen from hydrogen peroxide it would be difficult to account for the direct value of this additional oxygen for respirational processes in the presence of an abundance of atmospheric oxygen. If, however, the oxygenase-catechol system is accepted as an effective oxidation mechanism, then catalase would bear a necessary but indirect relation to the respiratory process.

Oxygenase-catechol-peroxidase.—It is also possible to set up a series of equations showing an oxygenase-catechol-peroxidase system. The chromogen formed by reaction 6 might be restored by the action of peroxidase and the peroxide formed by reactions 1 or 2.

7. 

This would not, of course, prevent the chromogen being oxidized also by equation 5. By this system it is possible to account not only for the lack of proportionality between catalase and respirational intensity as has been reported by Ranjan

and Mallik ('31), Rhine ('24), Harvey ('24), and McLeod and Gordon ('23), but also to explain the coordinate rise of both oxygenase and peroxidase in the heightened respirational rate of plants suffering from disease. A correlation between the oxygenase and peroxidase under pathological condition has been often reported. Suzuki ('00, '02 a) observed this condition in the mulberry dwarf disease in Japan; Woods ('02) reported it for the mosaic disease of tobacco; and Reed ('12) found that the extract from apples infected with bitter-rot (*Glomerella rufomaculans*) showed an increased activity of these enzymes. Doby ('11, '12) observed the same condition in potato tubers infected by the Rosette disease.

Invertase-peroxidase.—A third relation may be also postulated which involves a relationship between invertase and peroxidase. Since this theory places great importance on the recent work of Ranjan and Mallik ('31), their work will be reported in some detail. They found that the edible pea during germination exhibits a gradual rise of respiration, while the catalase falls slightly at first, then rises to a maximum, and again falls. The leaves of *Magnifera indica* showed a parallel decrease in respiration and catalase until yellowing set in. At this time catalase increased greatly, showing that the correlation existed only in the younger stages. The same results were found with the leaves of *Eugenia jambolana.* An analysis of the sugar content of the leaves of both these species showed that the curves of monosaccharide content almost exactly paralleled those of catalase activity.

In order to ascertain whether the sugar controlled the catalase or the catalase controlled the sugar, the stems of *Allium tuberosum* were injected with sugar, and some of the plants put in the light and others in the dark. The following important relation was discovered. It was not the hexose *presence* but the *formation* of hexose, either by photosynthesis or by the hydrolysis of disaccharides, which correlated with a high catalase activity. The injection of cane sugar accompanied a greater catalase activity than did glucose, although the amount of glucose present in the former case was related to the amount of catalase.

The apparent correlation between catalase and respiration as reported by other authors is explained by the fact that normally the formation of monosaccharides parallels respirational activity. Hexoses are being formed in old leaves since the complex carbohydrates are being broken down for translocation, and this accounts for the increased catalase at this stage. It is further pointed out that high catalase may be associated with low respiration unless high respiration is associated with the formation of hexose.

The present author proposes to relate the above phenomenon to account for the existence of an invertase-sugar-peroxidase respirational mechanism in the tomato plant under the conditions of the experiment. Waldschmidt-Leitz ('29) points out that the only known substrate for catalase is hydrogen peroxide. Since this substance is not demonstrated in tissues, it follows that the action of catalase or peroxidase uses it as rapidly as formed. The work of Ranjan and Mallik may therefore be interpreted as indicating that hydrogen peroxide, or a peroxide group resembling it but not yet recognized, develops during the formation of hexose. This is particularly evident, since it has been widely observed that an enzyme develops more or less proportionally to the concentration of its substrate. Rhine ('24) believed that catalase developed in plant tissue proportionally to the need of the plant to overcome the toxic effects of hydrogen peroxide.

Without entering the discussion concerning the importance of the ratio of sucrose to hexose in plant tissue in its relation to photosynthesis, we may note that sucrose often accumulates in a great excess over hexose. Davis and Sawyer ('16) studied the potato, a plant closely related to the tomato, and observed that sucrose was greatly in excess of hexose, and concluded that it was therefore the first sugar of photosynthesis. The same relationship has been found in many plants.

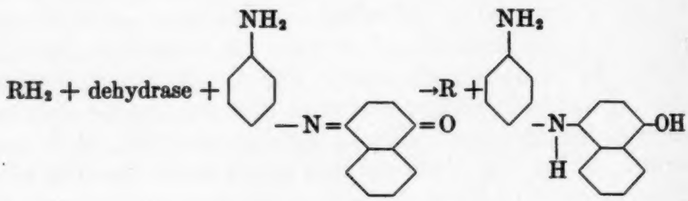
Regardless of whether sucrose is or is not the first sugar of photosynthesis, it is important for our hypothesis to note its predominance over hexose in plants closely related to the tomato. Onslow ('31) reviewed the literature concerning the substrate for respiration, and shows that most of the data

support the belief that a hexose, probably fructose, is this substrate. An increased rate of respiration would therefore call forth an increased invertase action to produce the hexose from sucrose. Since catalase has been shown to parallel the formation of hexose, it may be assumed that a transitory peroxide also develops simultaneously. Peroxidase might well use the peroxide then formed in the usual oxidation of chromogens. The comparatively small catalase increase might be thought of as a safety reaction destroying the peroxide in excess of the peroxide needs. The reaction may be represented as follows:

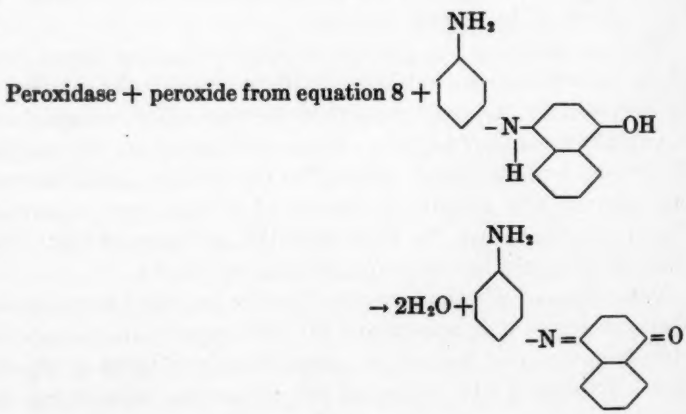
8. Sucrose + invertase → glucose + fructose + peroxide group.

9. Hexose + anaerobic enzymes → fermentation products.

10. Fermentation products indicated as RH_2



11.



An examination of the curves for respiration, and enzymatic activity in the present work shows interesting relationships. The increase in respiration is paralleled by peroxidase and diastase. Catalase also increases but not conspicuously, while oxygenase actually decreases.

The divergence of the respiration and oxygenase curves shows clearly that any such mechanism as the oxygenase-catechol system does not account for the increased respirational activities in this case.

The divergence of the peroxidase and oxygenase curves shows that the oxygenase-catechol-peroxidase system is not functioning.

The close correlation between the curves for respiration, invertase, and peroxidase indicates the possibility of the invertase-peroxidase series of reactions, and that this may be the mechanism for the increased respiration of the tomato plants grown in high potassium iodide solution. It seems probable that plants, such as horse-radish (*Cochlearia Armoracia*), which contain peroxidase but no oxygenase, might also depend on the invertase-peroxidase association. Certainly the possession of a strong peroxidase activity would be useless to a plant unless peroxides were available, either from such a reaction as suggested above for invertase or arising spontaneously by a non-enzymatic reaction.

V. Conclusions

1. Potassium iodide, in the concentrations used, exerted only a depressing influence upon growth.

2. Toxic effects consisted of a loss of green color and a progressive dropping of the lower leaves. No definite spotting of the leaves was observed.

3. No significant change in the acidity of the expressed sap was observed. The pH remained very close to 5.9. This prohibited the possibility of increased enzymatic activity being due to a more favorable pH of the sap.

4. Respiration, peroxidase, and invertase were decreased by a low concentration of potassium iodide (1 p.p.m.), but

greatly increased at the higher concentration. This increase does not parallel the degree of iodine injury. The increase of catalase at the higher concentrations was much less than that of respiration, peroxidase, or invertase.

5. Oxygenase activity is progressively lowered in the plants growing in higher potassium iodide concentrations, thereby differing from the other oxidizing enzymes studied. This is important evidence that the oxygenase-catechol system is not intimately concerned with the respiratory activity of the tomato plant.

6. The activity of peptase is not affected in the plants growing in different potassium iodide concentrations.

7. The stimulating effects observed on respiration and oxidizing activities of the injured plants are not similar to those induced by disease injury reported for other plants, since disease injury is known to increase also oxygenase.

8. A possible relationship of invertase to peroxidase and the effect of this relationship on respiration have been suggested, which may account for the increased respiration rate of the plant growing in the higher concentration of potassium iodide. This mechanism involves the formation of a transitory peroxide group during the formation of hexose from sucrose by the action of invertase. This peroxide may then be activated by peroxidase which oxidizes the hydrogen acceptor.

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Explanation of Plate

PLATE 17

The respiration chambers (see Wynd, '32).

WYND—RESPIRATION CHAMBERS